UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE VETERINARIA
Departamento de Nutrición, Bromatología y Tecnología de los Alimentos

TESIS DOCTORAL

Efecto de la composición y el tratamiento por altas presiones sobre el perfil de compuestos volátiles y la microbiota del jamón serrano e ibérico

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Nerea Martínez Onandi

Directores

Antonia Mª Picón Gálvez
Manuel Núñez Gutiérrez

Madrid, 2018

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE VETERINARIA

Departamento de Nutrición, Bromatología y Tecnología de los Alimentos

EFECTO DE LA COMPOSICIÓN Y EL TRATAMIENTO POR ALTAS PRESIONES SOBRE EL PERFIL DE COMPUESTOS VOLÁTILES Y LA MICROBIOTA DEL JAMÓN SERRANO E IBÉRICO

Memoria presentada por **Nerea Martínez Onandi** para la obtención del grado de Doctor por la Universidad Complutense de Madrid

Directores: Dra. Antonia Mª Picón Gálvez y Dr. Manuel Nuñez Gutiérrez

Departamento de Tecnología de Alimentos

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

Madrid, 2017

Antonia Mª Picón Gálvez, Científico Titular, y Manuel Nuñez Gutiérrez, Profesor de Investigación, del Departamento de Tecnología de Alimentos del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA),

CERTIFICAN:

Que la tesis titulada "Efecto de la composición y el tratamiento por altas presiones sobre el perfil de compuestos volátiles y la microbiota del jamón Serrano e Ibérico" de la que es autora Nerea Martínez Onandi, ha sido realizada bajo su dirección en el Departamento de Tecnología de Alimentos del INIA, y cumple las condiciones exigidas para optar al grado de Doctor por la Universidad Complutense de Madrid.

Madrid, 7 de Septiembre de 2017

Fdo. Antonia Mª Picón Gálvez

Fdo. Manuel Nuñez Gutiérrez

AGRADECIMIENTOS

No puedo creer que finalmente haya llegado el día… Y no me gustaría perder la oportunidad dar las gracias a todas aquellas personas que me acompañaron durante este intenso viaje:

A la Dra. Antonia Picón, directora de esta tesis, por la oportunidad que me brindó, su confianza, el interés mostrado, por todo lo que he aprendido de ella personal y profesionalmente, sinceramente ha sido un verdadero placer trabajar contigo mano a mano. Al Dr. Manuel Nuñez, co-director de esta tesis, sin ti y tu sabiduría nada de esto hubiese sido posible, gracias por tus palabras de ánimo en los momentos más duros.

Al ministerio de Economía y Competitividad por la concesión de una beca de formación de Personal Investigador (FPI). Esta Tesis Doctoral se ha realizado dentro del proyecto de investigación titulado: Optimización y control de la calidad tecnológica, nutricional y organoléptica de jamones Serranos e Ibéricos. RTA 2010-00029-C04-04.

Tante grazie a la Dra. Sandra Torriani, dell´Università di Verona, per avermi concesso essere nel tuo laboratorio, l´opportunità di imparare nuove tecniche e la tua disponibilità per consultare i dubbi. Ringrazio anche a Anna, per la tua gentilezza e ospitalità e per avermi insegnato benissimo. A la mia amica Valentina per il el tuo cuore, per farmi ridere tantísimo, e per avermi fatto sentire come a casa. A la resta de amici Arianna, Stefano, Marta, Roberta, Sara, Alex, Veronica, Fabio… grazie a tutti per il belli tempi. E´stata un´esperienza indimenticablei!!!!!

I would also like to thank to my foreign supervisors Dr Anne Maria Mullen and Dr Carlos Álvarez, (Food Chemistry and Technology Department, Division of Food Chemistry, Teagasc Ashtown Food Research Centre, Dublin) for welcoming me in their laboratory and research group and making me feel at home during my stay abroad. Both of you are outstanding people and two excellent academic supervisors. I would also like to thank all the mates and colleagues, particularly to my Spanish friends Cristina, José and Tomas, to my French friends Marion and Amelie for their hospitality and friendship. To one of my best friend Cristina B for your support in and out of the Lab. Thanks to all of you guys!

A todos mis compañeros del Departamento de Tecnología de Alimentos Pilar, Juan, Chema, Izaskun, Ana, Javi, Iria, Marta, Sonia, Rakel, María, Joaquin, Marga, Natalia, Elma, Paqui, Ana GI, Tomi y Ana Rivas por el cordial ambiente de trabajo, por vuestra ayuda, y por los buenos momentos compartidos con todos vosotros. A Sagrario, Pilar y Dani gracias por vuestros mejores consejos y

compañerismo. A Elvira por toda su ayuda con "la micro". Especialmente quiero agradecer a Olga López y Lucía Robles toda su paciencia por haberme escuchado, comprendido y ayudado en cada momento, porque vuestra generosidad y compañerismo la llevare siempre en mi corazón. ¡Os quiero chicas!

A la Dra. Mónica Flores, por introducirme en el maravilloso mundo de los volátiles y contagiarme las ganas de seguir aprendiendo cada día más sobre el tema. A todo el grupo de Ciencia de la Carne y, muy especialmente a la Dra. Leticia Mora y Dra. Elisabeth Escudero, por haberme acogido tan bien desde el primer día.

Al Dr. Pere Gou, Dra. Elena Fulladosa y Dr. Jacint Arnau, por toda vuestra ayuda ¡muchísimas gracias!.

A Marc y Laura, compañeros de proyecto, por esas risas siempre que hemos coincidido.

A mí querido profesor Antonio (Askartza Claret) porque tú fuiste la persona que más me ayudó a creer en mi misma y en mis posibilidades. Gracias por todas esas palabras que dejaste en mi corazón y que siempre recordaré.

A mi profesora y amiga Esther, como siempre te digo no tendré vida suficiente para agradecerte todo lo que has hecho por mí. Eres una persona generosa, bondadosa y amiga de tus amigas, ¡que más se puede pedir! Me considero una persona tremendamente afortunada por tenerte cerca. Thank you so much!!!

A Mari, dejaste un vacío inmenso en mi corazón cuando nos dejaste…todavía hoy cuando subo las escaleras pienso que puedo ver tu sonrisa y charlar un rato contigo como solíamos hacer. Allí donde estés no olvides todo lo que te quiero. ¡Gracias por todos los buenos momentos, gracias por hacerme una niña feliz!

A mis mejores amigos T-, R- y P- siempre os lo digo, y nunca me cansaré de deciros, que sois y seréis mi pilar. Gracias por estar siempre a mi lado.

A mi familia política, Valentín, Pili y Elisa, porque sin vosotros nada de esto hubiese sido igual. Gracias por vuestro cariño y comprensión, por estar siempre a nuestro lado y darnos los mejores consejos. A Valentín, por ser el mayor ejemplo de profesionalidad y perseverancia que una persona puede tener como referencia. Por ser el ángel que ilumina nuestro camino.

A mis hermanos, Silvia y Ramón. Tete nunca podré agradecerte todo lo que has hecho por mí. Tú has superado con creces lo que la palabra hermano significa, gracias por ser mi mejor amigo, escucharme y darme siempre los mejores consejos con esa risa que te caracteriza. You are the best BRO!

A marti, Carlitos y Paco por toda vuestra ayuda. Con gente así da gusto vivir y compartir la vida. ¡Gracias family!

Especialmente me veo en la obligación de agradecer todo esto a mi madre, una maravillosa madre que ha luchado por sus hijos muchísimo. Gracias por haberme dado los mejores consejos, por ser mi mejor amiga y confidente y por haberme inculcado que, con trabajo, todo se puede conseguir. Gracias por la mejor herencia que a unos hijos les puedes dejar, la educación. A mis aitites porque, allí donde estéis, sé que estaréis muy orgullosos de vuestra nieta.

A Andrés, gracias por escuchar mis pensamientos, por comprender mis sueños, por convivir con mis defectos y por quererme todos estos años. Eres la mitad que siempre soñé tener a mi lado ¡gracias por ser mi mejor complemento!, Te quiero.

GRACIAS A TODOS POR COMPARTIR ESTE VIAJE CONMIGO.

A mi mitad: Andrés.

Índice

Página

Resumen

Resumen

Los procesos de elaboración de jamón Serrano y jamón Ibérico constan de las mismas etapas de salado, post-salado y secado o maduración, precedidas de un acondicionamiento previo consistente en la recepción, clasificación y pre-salado de los perniles. Sin embargo, existen claras diferencias entre los dos tipos de jamones debidas a la raza, alimentación, manejo del animal, particularidades del proceso de elaboración y duración del periodo de maduración.

Entre los atributos de calidad más importantes en el jamón curado destaca su aroma intenso y persistente, debido a compuestos volátiles de bajo umbral de detección generados fundamentalmente durante la etapa de maduración. Entre las principales reacciones bioquímicas que tienen lugar en el jamón a lo largo de su elaboración y maduración destacan la proteolisis, lipólisis, y en menor medida, la glucólisis y la degradación de nucleótidos. Otras reacciones secundarias tales como reacciones de Maillard, degradación de Strecker y oxidación de lípidos y proteínas tienen lugar con los productos de la hidrólisis primaria como sustratos.

El tratamiento de altas presiones hidrostáticas (APH), clasificado como una pasteurización no térmica y de procesado mínimo, es una de las tecnologías emergentes para el procesado de alimentos que más ha crecido en los últimos años. A diferencia de otras técnicas de conservación, genera una percepción positiva por parte del consumidor. Su principal objetivo es la inactivación de microorganismos patógenos y alterantes y de enzimas para aumentar la seguridad y la estabilidad del alimento. Por ello, resulta muy útil para las empresas exportadoras de productos cárnicos envasados. El tratamiento de la carne y los productos cárnicos por APH puede afectar a algunas de sus características relacionadas con la calidad organoléptica, tales como la textura, el color y el nivel de oxidación lipídica. En el caso concreto del jamón curado, se han llevado a cabo diversos estudios sobre

el efecto de las APH en la microbiología, el color, la textura, las actividades enzimáticas, la oxidación lipídica, los compuestos químicos y las características sensoriales.

Los objetivos de la presente Tesis Doctoral fueron investigar la influencia de la composición química y las altas presiones hidrostáticas (APH) sobre los compuestos volátiles y la microbiota presentes en jamón Serrano y jamón Ibérico madurados, a los 3 días de la aplicación del tratamiento y después de 5 meses de almacenamiento en refrigeración a 4 ºC.

En la fracción volátil del jamón Serrano madurado a los 3 días de refrigeración se identificaron 100 compuestos. Su composición química afectó principalmente a algunos ácidos, alcoholes, aldehídos ramificados, cetonas, compuestos bencénicos, compuestos azufrados, furanos, furanonas y pirazinas. El contenido de grasa intramuscular fue el parámetro composicional que más influyó en la fracción volátil del jamón Serrano, afectando a los niveles de 51 compuestos volátiles, principalmente compuestos procedentes de reacciones de oxidación lipídica, de reacciones de Maillard, de la degradación de Strecker, del catabolismo de aminoácidos azufrados y del metabolismo microbiano.

En la fracción volátil del jamón Serrano madurado después de 5 meses en refrigeración se identificaron 103 compuestos. Su composición química afectó principalmente a algunos ácidos, alcoholes, aldehídos, alcanos, cetonas, compuestos bencénicos, compuestos azufrados, ésteres y furanos. El contenido de grasa intramuscular fue igualmente el parámetro composicional que más influyó sobre la fracción volátil del jamón Serrano al final del periodo de refrigeración, afectando a los niveles de 46 compuestos volátiles, principalmente compuestos procedentes de reacciones de oxidación lipídica, de reacciones de Maillard, de la reacción de esterificación enzimática y del metabolismo microbiano.

El tratamiento de APH afectó significativamente a los niveles de 8 de los 100 compuestos volátiles identificados en jamón Serrano madurado a los 3 días de la aplicación del tratamiento y a los niveles de 21 de los 103 compuestos volátiles identificados después de 5 meses en refrigeración.

De los 108 compuestos identificados en la fracción volátil del jamón Serrano, 95 se detectaron tanto al principio como al final de la refrigeración, 5 solamente al principio de la refrigeración y 8 solamente al final de la refrigeración. En las muestras control de jamón Serrano, los niveles totales de alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanonas, terpenos y compuestos misceláneos disminuyeron durante la refrigeración. En las muestras de jamón Serrano tratadas por APH los niveles totales de alcoholes, cetonas, terpenos y compuestos misceláneos disminuyeron y los niveles totales de furanos aumentaron durante la refrigeración.

La composición química del jamón Serrano a los 3 días de refrigeración influyó sobre los niveles de aerobios mesófilos, psicrotrofos, bacterias lácticas y mohos y levaduras. La concentración de sal fue el parámetro composicional que más influyó sobre los niveles de microorganismos en el jamón Serrano a los 3 días de refrigeración, afectando a aerobios mesófilos, psicrotrofos, bacterias lácticas y mohos y levaduras en las muestras control.

La composición química del jamón Serrano después de 5 meses en refrigeración influyó sobre los niveles de aerobios mesófilos, psicrotrofos, micrococáceas y mohos y levaduras. El contenido en grasa intramuscular fue el parámetro composicional que más influyó sobre los niveles de microorganismos en el jamón Serrano después de 5 meses en refrigeración, afectando a aerobios mesófilos, psicrotrofos, micrococáceas y mohos y levaduras en las muestras control.

El tratamiento por APH del jamón Serrano redujo significativamente los niveles de todos los grupos microbianos estudiados. Al cabo de 5 meses en refrigeración, se observó en las muestras tratadas por APH una recuperación de los microorganismos hasta niveles similares a los de las muestras control, con la única excepción de las bacterias lácticas.

La microbiota del jamón Serrano madurado a los 3 días de refrigeración determinada por DGGE estaba compuesta por las especies bacterianas *Staphylococcus equorum*, *S. succinus* y *Bacillus subtilis*, así como por las especies eucariotas *Penicillium commune*, *P. chrysogenum*, *Aspergillus fumigatus*, *Sclerotinia sclerotiorum*, *Eurotium athecium*, *Moniliella mellis*, *Debaryomyces hansenii* y *Candida glucosophila*. El tratamiento de APH produjo ligeros cambios en la población microbiana, puestos de manifiesto por la desaparición de las bandas de *B. subtilis* y la atenuación de las bandas de *E. athecium*.

En la fracción volátil del jamón Ibérico madurado a los 3 días de refrigeración se identificaron 122 compuestos. Su composición química afectó principalmente a algunos ácidos, alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados y furanonas. El contenido de grasa intramuscular fue el parámetro composicional que más influyó sobre la fracción volátil del jamón Ibérico, afectando a 20 compuestos volátiles, principalmente compuestos procedentes de reacciones de oxidación lipídica y de la alimentación de los animales.

En la fracción volátil del jamón Ibérico madurado después de 5 meses en refrigeración se identificaron 116 compuestos. Su composición química afectó principalmente a algunos ácidos, alcoholes, aldehídos, alcanos y compuestos bencénicos. El contenido en grasa intramuscular fue el parámetro composicional que más influyó en la fracción volátil del jamón Ibérico, afectando a 5 compuestos volátiles, principalmente compuestos procedentes del metabolismo microbiano.

El tratamiento de APH influyó sobre los niveles de 35 de los 122 compuestos volátiles identificados en el jamón Ibérico madurado a los 3 días de la aplicación del tratamiento y sobre 34 de los 116 compuestos volátiles identificados después de 5 meses en refrigeración.

De los 129 compuestos identificados en la fracción volátil del jamón Ibérico, 109 se detectaron tanto al principio como al final de la refrigeración, 13 solamente al principio de la refrigeración y 7 solamente al final de la refrigeración. En las muestras control de jamón Ibérico, los niveles totales de ácidos, alcoholes, cetonas, ésteres, compuestos azufrados, furanos, furanonas, pirazinas y compuestos

misceláneos disminuyeron y los niveles totales de los compuestos bencénicos aumentaron durante el periodo de refrigeración. En las muestras de jamón Ibérico tratadas por APH los niveles totales de ácidos, alcoholes, cetonas, ésteres, furanos, furanonas y pirazinas disminuyeron y los niveles totales de compuestos bencénicos, compuestos azufrados y compuestos misceláneos aumentaron durante la refrigeración.

La composición química del jamón Ibérico a los 3 días de refrigeración influyó sobre los niveles de psicrotrofos, micrococáceas y bacterias lácticas. La a_w fue el parámetro composicional que más influyó en los niveles de microorganismos en jamón Ibérico, afectando a los niveles de psicrotrofos y micrococáceas en las muestras control.

La composición química del jamón Ibérico después de 5 meses en refrigeración influyó sobre los niveles de mohos y levaduras. El contenido en grasa intramuscular fue el único parámetro composicional que influyó en los niveles de microorganismos en jamón Ibérico, afectando a los niveles de mohos y levaduras en las muestras tratadas por APH.

El tratamiento por APH redujo los niveles de todos los grupos microbianos estudiados en jamón Ibérico. Al cabo de 5 meses en refrigeración, se observó en las muestras tratadas por APH una cierta recuperación de los microorganismos aunque los niveles de todos los grupos microbianos estudiados se mantuvieron significativamente más bajos que los de las respectivas muestras control.

La microbiota del jamón Ibérico madurado estaba compuesta mayoritariamente por bacterias del género *Staphylococcus*, con *S. equorum* como especie predominante, y levaduras de la especie *Debaryomyces hansenii*. Entre los aislados bacterianos se detectó la presencia de los géneros *Tetragenococcus* y *Carnobacterium*, que no habían sido detectados anteriormente en jamón curado.

Summary

Summary

The manufacturing process of Serrano and Iberian dry-cured hams is composed of the same stages (salting, post-salting and drying or maturation), preceded by a previous conditioning consisting in the reception, classification and pre-salting of the legs. However, clear differences between both types of ham are due to breed, feeding, animal handling and some variations in the manufacturing process and ripening length.

The intense and persistent aroma, one of the most important sensory characteristics of dry-cured ham, is due to the low detection threshold volatile compounds generated during the ripening stage. During manufacture and ripening, the main biochemical reactions that take place are proteolysis, lipolysis and to a lesser extent, glycolysis and nucleotide degradation. The products generated by those reactions serve as substrate of secondary reactions such as Maillard reactions, Strecker degradation together with lipid and protein oxidation reactions.

High pressure processing (HPP), classified as non-thermal and minimal processing pasteurization, is one of the emerging technologies for food processing which has experienced a huge growth in the last years. Unlike other preserving techniques, it is well accepted by consumers. Its main objective is to inactivate pathogenic and spoilage microorganisms and enzymes, ensuring food safety and increasing shelf-life. However, the HPP-treatment of meat and meat products may alter some of their organoleptic characteristics, such as texture, colour and lipid oxidation level. In the specific case of dry-cured ham, several studies on the effect of HPP on microbial levels, colour, texture, enzymatic activities, lipid oxidation, chemical compounds and sensory characteristics have been carried out.

The objectives of this Ph. D. thesis were to investigate the influence of the chemical composition and HPP on the volatile profile and microbiota of ripened Serrano and Iberian hams, after 3 days and 5 months of refrigerated storage at 4 ºC.

One hundred compounds were detected in the volatile fraction of ripened Serrano ham after 3 days of refrigerated storage. Its chemical composition mainly affected some acids, alcohols, branched-chain aldehydes, ketones, benzene compounds, sulfur compounds, furans, furanones and pyrazines. Intramuscular fat content was the compositional parameter with the highest influence on Serrano ham volatile fraction after 3 days of refrigerated storage. It affected the levels of 51 volatile compounds, mainly derived from lipid oxidation reactions, Maillard reactions, Strecker degradation, catabolism of sulfur amino acids and microbial metabolism.

One hundred and three compounds were detected in the volatile fraction of ripened Serrano ham after 5 months of refrigerated storage. Its chemical composition mainly affected some acids, alcohols, aldehydes, alkanes, ketones, benzene compounds, sulfur compounds, esters and furans. Intramuscular fat content was also the compositional parameter with the highest influence on Serrano ham volatile fraction after 5 months of refrigerated storage. It affected the levels of 46 volatile compounds, mainly derived from lipid oxidation reactions, Maillard reactions, enzymatic esterification reactions and microbial metabolism.

HPP treatment significantly affected the levels of 8 out of the 100 volatile compounds identified in ripened Serrano ham after 3 days of refrigerated storage and of 21 out of the 103 volatile compounds identified after 5 months of refrigerated storage.

Out of the 108 compounds identified in the Serrano ham volatile fraction, 95 were detected both at the beginning and the end of the refrigerated storage, 5 were only detected at the beginning and 8 only at the end of the refrigerated storage. In Serrano ham control samples, the total levels of alcohols, aldehydes, ketones, benzene compounds, sulfur compounds, furanones, terpenes and miscellaneous

compounds decreased during the refrigerated storage. In HPP-treated Serrano ham samples, total levels of alcohols, ketones, terpenes and miscellaneous compounds decreased and total levels of furans increased during the refrigerated storage.

Serrano ham chemical composition after 3 days of refrigerated storage influenced the levels of aerobic mesophiles, psychrotrophs, lactic acid bacteria, moulds and yeasts. Salt concentration was the compositional parameter with the highest influence on Serrano ham microbial levels, affecting aerobic mesophiles, psychrotrophs, lactic acid bacteria and moulds and yeasts in control samples.

Serrano ham chemical composition after 5 months of refrigerated storage influenced the levels of aerobic mesophiles, psychrotrophs, *Micrococcaceae*, moulds and yeasts. Intramuscular fat content was the compositional parameter with the highest influence on Serrano ham microbial levels after 5 months of refrigerated storage, affecting the levels of aerobic mesophiles, psychrotrophs, *Micrococcaceae*, moulds and yeasts in control samples.

Serrano ham HPP-treatment significantly lowered the levels of all studied microbial groups. However, after a 5-month refrigeration period, microbial levels in HPP-treated samples were similar to those of control samples, with the only exception of lactic acid bacteria.

The microbiota of ripened Serrano ham after 3 days of refrigerated storage, analysed by DGGE, was composed of the bacterial species *Staphylococcus equorum*, *S. succinus* and *Bacillus subtilis*, and the eukaryotic species *Penicillium commune*, *P. chrysogenum*, *Aspergillus fumigatus*, *Sclerotinia sclerotiorum*, *Eurotium athecium*, *Moniliella mellis*, *Debaryomyces hansenii* and *Candida glucosophila*. Slight changes in the microbial population were observed after HPP-treatment, evidenced by the disappearance of *B. subtilis* bands and the attenuation of *E. athecium* bands.

One hundred and twenty-two compounds were identified in the volatile fraction of ripened Iberian after 3 days of refrigerated storage. Its chemical composition mainly affected some acids, alcohols, aldehydes, ketones, benzene compounds, sulfur compounds and furanones. Intramuscular fat

content was the compositional parameter with the highest influence on Iberian ham volatile fraction after 3 days of refrigerated storage. It affected the levels of 20 volatile compounds, mainly derived from lipid oxidation reactions and animal feed.

One hundred and sixteen compounds were identified in the volatile fraction of ripened Iberian ham after 5 months of refrigerated storage. Its chemical composition mainly affected some acids, alcohols, aldehydes, alkanes and benzene compounds. Intramuscular fat content was the compositional parameter with the highest influence on Iberian ham volatile fraction after 5 months of refrigerated storage. It affected the levels of 5 volatile compounds, mainly derived from microbial metabolism.

HPP treatment significantly affected the levels of 35 out of the 122 volatile compounds identified in Iberian ham after 3 days of refrigerated storage and of 34 out of the 116 volatile compounds identified after 5 months of refrigerated storage.

Out of the 129 compounds identified in the Iberian ham volatile fraction, 109 were detected both at the beginning and at the end of the refrigerated storage, 13 were only detected at the beginning and 7 only at the end of the refrigerated storage. In Iberian ham control samples, the total levels of acids, alcohols, ketones, esters, sulfur compounds, furans, furanones, pyrazines and miscellaneous compounds decreased and the levels of benzene compounds increased during the refrigeration period. In Iberian ham HPP-treated samples, the total levels of acids, alcohols, ketones, esters, furans, furanones and pyrazines decreased and the total levels of benzene compounds, sulfur compounds and miscellaneous compounds increased during the refrigeration period.

Iberian ham chemical composition after 3 days of refrigerated storage influenced the levels of psychrotrophs, *Micrococcaceae* and lactic acid bacteria. The compositional parameter with the highest influence on Iberian ham microbial population was a_w. It affected the levels of psychrotrophs and *Micrococcaceae* in control samples.

Iberian ham chemical composition after 5 months of refrigerated storage influenced the levels of moulds and yeasts. Intramuscular fat content was the only compositional parameter that influenced Iberian ham microbial levels, affecting moulds and yeasts levels in HPP-treated samples.

Iberian ham HPP-treatment decreased the levels of all studied microbial groups. After 5 months of refrigerated storage, a significant recovery in all microbial groups was observed in HPP-treated samples, although levels were still lower than those in untreated hams.

Iberian ham microbiota was mainly composed of bacterial isolates belonging to the genus *Staphylococcus*, with S. *equorum* as the predominant species, and yeast isolates of the species *Debaryomyces hansenii*. Isolates belonging to the genera *Tetragenococcus* and *Carnobacterium* isolates, which had not been previously reported in dry-cured ham, were also detected.

Listado de abreviaturas

Listado de abreviaturas / Abbreviation list

a*	Componente rojo-verde de las variables de color CIE (L*, a*, b*) / Red- green component of CIE color variables (L *, a *, b *)
a_w	Actividad de agua / Water activity
amu	Unidad de masa atómica / Atomic mass unit
ANOVA	Análisis de la varianza / Analysis of variance
AOAC	Asociación de Químicos Analíticos Oficiales / Association of Official Analytical Chemists
APH	Altas presiones hidrostáticas / High hydrostatic pressure
ARDRA	Análisis de restricción de ADN ribosomal amplificado / Amplified ribosomal DNA restriction analysis
*Ava*II	Endonucleasa *Ava*II, aislada de / Endonuclease *Ava*II, isolated from *Anabaena variabilis*
b*	Componente amarillo-azul de las variables de color CIE (L*, a*, b*) / Yellow-blue component of CIE color variables (L *, a *, b *)
BCA	Alcanos ramificados / Branched-chain alkanes
BLAST	Base de datos / Database 'Basic Local Alignment Search Tool'
bp	Pares de bases / Base pairs
BP	Agar Baird-Parker / Baird-Parker agar
BSA	Agar brilliance Salmonella / Brilliance Salmonella agar
C	Muestra control / Untreated control sample
C5-C20	Serie de alcanos con cadenas de 5 a 20 átomos de carbono / Series of alkanes with 5 to 20 carbon atoms in length
cfu	Unidades formadoras de colonia / Colony forming units

ºC	Grados centígrados / Celsius degrees
DGGE	Electroforesis en gel con gradiente de desnaturalización / Denaturing gradient gel electrophoresis
DNA	Ácido desoxirribonucleico / Deoxyribonucleic acid
dNTP	Desoxinucleótido trifosfato / Deoxynucleotide triphosphate
DS	Desviación estándar / Standard deviation
DVB/CAR/PDMS	Divinilbenceno/carboxen/polidimetilsiloxano / Divinylbenzene/carboxen/ polydimethylsiloxane
EDTA	Ácido etilendiaminotetraacético / Ethylendiaminetetraacetic acid
eV	Electronvoltio / Electronvolt
g	Gramos / Grams
GC	Cromatografía de gases / Gas chromatography
GC-MS	Cromatografía de gases-espectrometría de masas / Gas chromatography-mass spectrometry
h	Horas / Hours
HPP	Procesado por altas presiones / High pressure processing
ID	Identificación del pico / Peak identification
IMF	Grasa intramuscular / Intramuscular fat
INIA	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria / National Institute of Agricultural and Food Research and Technology
IRTA	Instituto de Investigación y Tecnología Agroalimentarias / Institute of Food and Agricultural Research
ITS	Espaciador interno transcrito / Internal transcribed spacer
KAA	Kanamicina esculina azida / Kanamycin aesculin azide
kg	Kilogramos / Kilograms

*Kpn*I	Endonuclease *Kpn*I, aislada de / Endonuclease *Kpn*I, isolated from *Klebsiella pneumoniae*
L	Litros / Litres
L*	Luminosidad o claridad de las variables de color CIE (L*, a*, b*) / Brightness or clarity of CIE color variables (L *, a *, b *)
LAB	Bacterias lácticas / Lactic acid bacteria
log	Logaritmo / Logarithm
LRI	Índice de retención lineal / Linear retention index
M	Molar
M	Marcador / Marker
*Mbo*I	Endonucleasa *Mbo*I, aislada de / Endonuclease *Mbo*I, isolated from *Moraxella bovis*
mg	Miligramos / Milligrams
min	Minutos / Minutes
MKTTn	Muller-Kauffmann tetrationato novobiocina / Muller-Kauffmann tetrathionate novobiocin
ml	Mililitros / Millilitres
mm	Milímetros / Millimetres
MPa	Megapascales / Megapascals
MRS	Agar de Man, Rogosa y Sharpe / De Man, Rogosa and Sharpe agar
MS	Masa / Mass
MSA	Agar manitol sal / Mannitol salt agar
µl	Microlitros / Microlitres
µm	Micrometros / Micrometres
µM	Micromolar

n	Número / Number
NCBI	Centro Nacional de Información Biotecnológica / National Center for Biotechnology Information
ng	Nanogramos / Nanograms
ns	no significativo / non-significant
P	Significación estadística según ANOVA / Statistical significant in the ANOVA
PCA	Agar para recuento en placa / Plate count agar
PCA	Análisis de componentes principales / Principal component analysis
PCR	Reacción en cadena de la polimerasa / Polymerase chain reaction
PTFE	Politetrafluoroetileno / Polytetrafluoroethylene
QI	Iones utilizados para la cuantificación / Ions used for quantification
RDP	Base de datos / Database 'Ribosomal Database Project'
RH	Humedad relativa / Relative humidity
RPF	Fibrinógeno de plasma de conejo / Rabbit plasma fibrinogen
rRNA	Ácido ribonucleico ribosómico / Ribosomal ribonucleic acid
RVS	Rappaport Vassiliadis soja / Rappaport Vassiliadis soy
s	Segundos / Seconds
SD	Desviación estándar / Standard deviation
SDA	Agar Sabouraud dextrosa / Sabouraud dextrose agar
SE	Error estándar de la media / Standard error of the mean
SPME	Microextracción en fase sólida / Solid phase microextraction
spp.	Especies / Species
SPSS	Paquete estadístico / Statistical package SPSS
ST	Estándar / Standard
S / L	Relación sal en magro / Salt in lean ratio

T	Tratado por altas presiones / High pressure-treated
TAE	Tris-acetato-EDTA / Tris-acetate-EDTA
Taq	Polimerasa, aislada de / Polymerase, isolated from *Thermus aquaticus*
*Taq*I	Endonucleasa *Taq*I, aislada de / Endonuclease *Taq*I isolated from *Thermus aquaticus*
TE	Tris-EDTA / Tris-EDTA
U	Unidades / Units
ufc	Unidades formadoras de colonia / colony forming units
UV	Ultravioleta / Ultraviolet
UVMI	Caldo de enriquecimiento de *Listeria* / *Listeria* enrichment broth
V	Voltios / Volts
VRBG	Bilis rojo violeta glucosa / Violet red bile glucose
XLD	Xilosa lisina desoxicolato / Xylose lysine deoxycholate
w/v / wt/vol	Relación peso:volumen / Weight:volume ratio

1. Introducción general

1.1. La elaboración de jamón Serrano e Ibérico

La elaboración de jamón curado ha constituido desde tiempo inmemorial una forma de conservación de los perniles de cerdo mediante su salado y el posterior secado. Actualmente, se persigue además la obtención de jamones de mayor valor añadido, seguros desde el punto de vista microbiológico y de una alta calidad nutricional y sensorial. El producto que se obtiene al final del proceso viene condicionado por la materia prima utilizada y por el proceso aplicado en cada industria. Existen distintas tecnologías de fabricación de jamón curado pero básicamente todas ellas tienen como objetivo conseguir un producto estable y seguro a temperatura ambiente y facilitar el correcto desarrollo de sus características sensoriales.

El proceso de elaboración del jamón curado está constituido por las etapas de salado, post-salado y secado o maduración, precedidas de un acondicionamiento previo consistente en la recepción, clasificación y pre-salado de los perniles (Toldrá et al., 1997). Este proceso de elaboración, válido en líneas generales tanto para jamón Serrano como para jamón Ibérico, se resume en la Figura 1. En España se producen unos 18 millones de jamones Serranos y unos 5 millones de jamones Ibéricos, aunque estos últimos sufren mayores oscilaciones de un año a otro. Existen claras diferencias entre el jamón Serrano y el jamón Ibérico, debidas a la raza de cerdo de que provienen, la alimentación y el manejo de los animales, algunas particularidades del proceso de elaboración y la duración del periodo de maduración. Estos factores confieren a cada tipo de jamón unas características organolépticas exclusivas.

Con el salado se pretende que el pernil adquiera un contenido de sal suficiente para inhibir el desarrollo de microorganismos alterantes y potencialmente patógenos para el consumidor, conseguir un ligero sabor salado en el producto final y regular la actividad enzimática endógena y las reacciones químicas durante la maduración.

En los países mediterráneos el salado de los jamones se realiza por el tradicional sistema de salado en seco. En el pre-salado los perniles son frotados superficialmente con una mezcla de sal común y sales nitrificantes (nitratos y nitritos), que proporcionan el color rojo estable debido a la formación de nitrosomioglobina, el pigmento característico del curado (Cheftel y Culioli, 1997; Clariana et al., 2011). Además, el nitrato inhibe el crecimiento de microorganismos anaerobios alterantes y potencialmente patógenos que pudieran contaminar el jamón tales como *Clostridium botulinum* (Sanabria et al., 1997). Junto con las sales nitrificantes pueden ir otros aditivos autorizados (antioxidantes, acidulantes y azúcares), generalmente empleados en otros tipos de jamón pero que apenas se utilizan en la elaboración de jamón Ibérico.

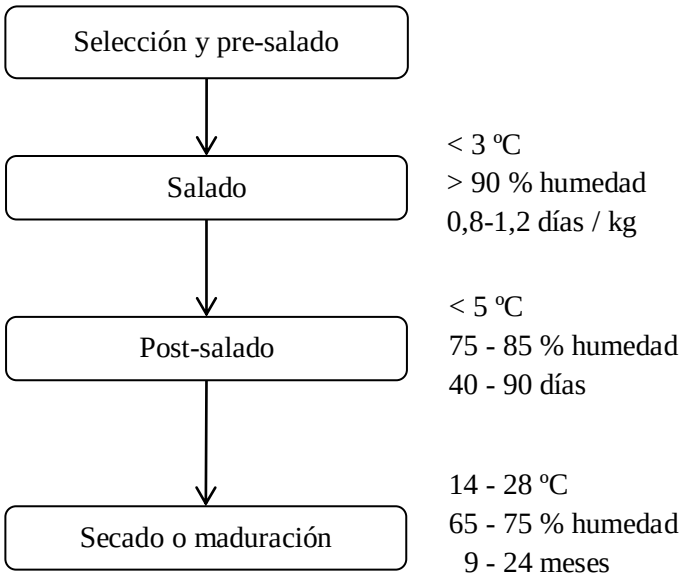


Figura 1. Diagrama de flujo del proceso de elaboración del jamón curado.

Posteriormente, los jamones son acondicionados en saladeros, colocados con la cara grasa hacia abajo, en capas alternativas de abundante sal y perniles. Durante el salado es importante controlar la temperatura y la humedad relativa. Para conseguir un equilibrio entre estos dos parámetros la temperatura se mantiene entre 0 y 3 ºC y la humedad relativa entre el 90 y el 95 %. A este nivel de humedad se hidrata la sal, lo que favorece su penetración en el pernil. La granulometría de la sal también es un parámetro importante, siendo más frecuente la utilización de sal de grano grueso. El

tiempo de permanencia de los perniles en las pilas de sal varía entre 0,8 y 1,2 días / kg de peso de la pieza (de 8 a 12 días para un pernil de 10 kg). Una vez acabado el periodo de salado, se elimina la sal sobrante de la superficie de los perniles mediante cepillado y lavado y se dejan escurrir en refrigeración durante un periodo de tiempo inferior a 24 h (Ventanas et al., 2001).

La finalidad de la etapa de post-salado es conseguir una distribución homogénea de la sal por todo el jamón al tiempo que una deshidratación adecuada del producto. Para que el secado sea correcto, la cantidad de agua que se evapora de la superficie debe estar compensada por la difusión gradual de agua del interior a la superficie del jamón. Esta etapa se lleva a cabo en cámaras frigoríficas a una temperatura entre 3 y 5 ºC y una humedad relativa en progresivo descenso desde el 85 hasta el 75 %. La duración de esta etapa varía en función del tamaño de la pieza, oscilando normalmente entre 40 y 60 días para jamón Serrano y entre 75 y 90 días para jamón Ibérico.

La etapa de secado o maduración tiene gran importancia en el proceso de elaboración del jamón curado. En esta etapa se pretende conseguir la estabilización total del jamón mediante la exposición progresiva a temperaturas más elevadas y humedades relativas más bajas. La elevación gradual de la temperatura hasta llegar a alcanzar un máximo de 22 a 28 ºC acompañada de un descenso de la humedad relativa hasta niveles del 65 % tiene por objeto seguir deshidratando el producto y disminuyendo su actividad de agua (a_w) así como favorecer la formación de los compuestos responsables del sabor y aroma del jamón, a través de reacciones que incluyen la proteolisis, la lipolisis, la oxidación de lípidos y la degradación secundaria de los productos de hidrólisis (García et al., 1991).

1.2. La maduración del jamón

Entre los atributos de calidad más importantes en el jamón curado destacan la intensidad del olor así como un aroma intenso y persistente. Estos atributos son debidos principalmente a un gran número de compuestos volátiles con bajo umbral de detección olfativa (Flores et al., 1997). Los compuestos

volátiles y no volátiles responsables del olor, aroma y sabor del jamón curado se generan fundamentalmente durante la etapa de maduración, que dura por lo general de 9 a 12 meses en jamón Serrano y de 18 a 24 meses en jamón Ibérico.

Las principales reacciones bioquímicas que tienen lugar en el jamón a lo largo de su elaboración y maduración son mayoritariamente de tipo enzimático, destacando la hidrólisis de proteínas musculares (proteolisis), la hidrólisis de triglicéridos y fosfolípidos (lipolisis) y, en menor medida, la hidrolisis de glúcidos (glucolisis) y la degradación de nucleótidos. Con los productos de hidrólisis como sustratos tienen lugar reacciones químicas secundarias tales como las reacciones de Maillard, la degradación de Strecker y la oxidación de lípidos y proteínas. Todas estas reacciones ocurren de manera simultánea y su intensidad depende de las condiciones durante la elaboración y maduración del jamón (Flores et al., 1997).

1.2.1. Proteolisis

Las principales proteínas estructurales del músculo (miosina, actina, titina o conectina y nebulina) sufren una importante degradación durante su conservación post-mortem, lo que confiere terneza a la carne (Lametsch et al., 2003). Durante la maduración del jamón estos procesos proteolíticos continúan. La proteolisis durante la maduración del jamón depende de las características de la carne, las cuales son a su vez función de factores biológicos como la edad y el sexo del animal, el tipo de músculo, el estado fisiológico del animal antes y durante el sacrificio, y las condiciones de procesado de la canal después del mismo, con efectos importantes sobre las enzimas musculares.

La proteolisis (Figura 2) es un proceso de naturaleza enzimática en el que están implicadas fundamentalmente enzimas musculares endógenas, que afectan tanto a las proteínas sarcoplásmicas como a las miofibrilares. El músculo esquelético contiene una gran variedad de enzimas proteolíticas, dentro de las cuales las endopeptidasas son responsables de la hidrólisis de las proteínas y las exopeptidasas de la generación de péptidos de bajo peso molecular y aminoácidos libres (Toldrá,

2006). Las principales enzimas proteolíticas asociadas a la hidrolisis de las proteínas son las calpaínas (μ-calpaína y m-calpaína), las catepsinas (B, D, H y L), el proteasoma y las caspasas.

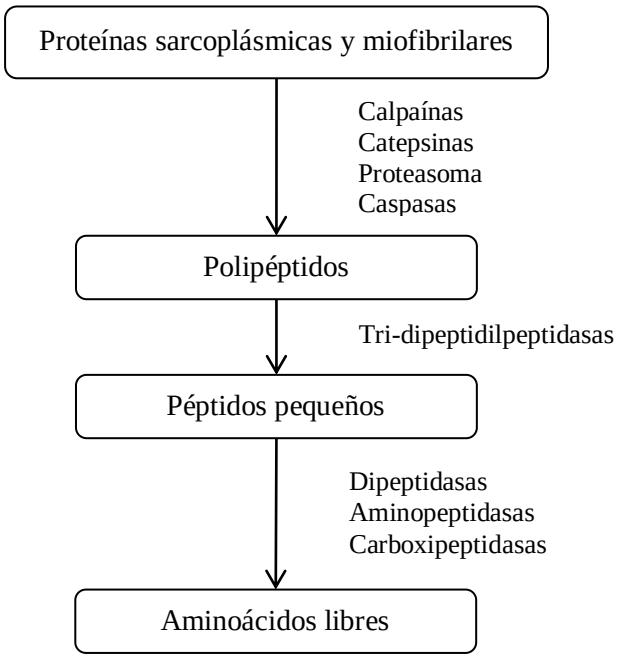


Figura 2. Etapas de la proteolisis durante el proceso de elaboración del jamón curado

Las calpaínas (sistema proteolítico Ca^{2+}-dependiente) son un grupo de cisteín-endopeptidasas que se encuentran en el citosol y, especialmente, en la región del disco Z. La μ-calpaína requiere entre 50 y 70 μM de Ca^{2+} para su activación y la m-calpaína entre 1 y 5 mM de Ca^{2+}. Ambas muestran su actividad máxima a pH neutro (alrededor de 7,5). Otro componente importante del sistema proteolítico es un inhibidor altamente específico de estas enzimas denominado calpastatina, que actúa a concentraciones de Ca^{2+} mayores que las necesarias para la activación de las calpaínas. Este inhibidor se activa por autolisis a los pocos días tras el sacrificio y regula la actividad de las calpaínas en el músculo post-mortem (Koohmaraie et al., 1987). En comparación con otras especies, el músculo de cerdo presenta niveles bajos de calpastatina (Ouali y Talmant, 1990). Se han observado mayores niveles de calpaínas en jamones de cerdo blanco que en los de cerdo ibérico (Rosell y Toldrá, 1998).

Sin embargo, se ha comprobado que al final del salado estás enzimas no están activas (Córdoba et al., 1994; Sárraga et al., 1989).

Las catepsinas son endopeptidasas localizadas en los lisomomas. Las catepsinas B, H, y L son cisteín-peptidasas mientras que la catepsina D es una aspartil-peptidasa. Son enzimas de pequeño tamaño que, una vez liberadas, penetran fácilmente en la estructura miofibrilar. La catepsina D es activa a pH ácido (pH 3 - 4), las catepsinas B y L a pH ligeramente ácido (alrededor de pH 6) y la catepsina H a pH neutro (pH 6,8) a 30 - 40 ºC (Rico et al., 1991a, b). En estudios realizados en jamón curado se ha observado que las catepsinas B, D, H y L permanecen activas incluso a los 8 meses de procesado (Toldrá y Etherington, 1988). La catepsina H es inhibida en gran medida por la sal durante la maduración mientras que el efecto inhibitorio de la sal es menor en el caso de las catepsinas B y L (Rico et al., 1991b). Otros autores no han encontrado una correlación entre las actividades de las catepsinas B, D o H y el contenido en sal del jamón (García-Garrido et al., 2000). La actividad de las catepsinas tiene mucha importancia durante el proceso de elaboración del jamón. Por un lado favorecen la liberación de péptidos de tamaño intermedio que servirán como sustrato de otras enzimas, y su actividad se ve favorecida por el aumento de la temperatura en la etapa de secado o maduración. Por otro lado, un exceso de actividad de las catepsinas, en el caso de que los jamones se sometan a una temperatura elevada y la concentración de sal en el interior sea todavía baja, puede dar lugar a un ablandamiento de la textura y a la formación de cristales grandes de tirosina en la superficie de corte del jamón. Se ha observado que en jamones con una textura defectuosa debida a una proteolisis excesiva los niveles de nitrógeno no proteico fueron más elevados y existía una correlación entre este parámetro y la actividad de las catepsinas B + L (García-Garrido et al., 2000)

El proteasoma es un complejo enzimático multicatalítico no localizado en los lisosomas, que puede hidrolizar enlaces peptídicos en los que participen aminoácidos hidrofóbicos, básicos o ácidos en su extremo carboxílico. Su pH óptimo de actividad se encuentra entre 7 y 9, dependiendo de la reacción que catalice (Djaballah y Rivett, 1992).

Las caspasas son un grupo de peptidasas pertenecientes al grupo de las cisteín-peptidasas, implicadas en el fenómeno de apoptosis post-mortem. Tienen una especificidad de sustrato limitada a la hidrólisis de enlaces peptídicos con ácido aspártico en extremo amino. La acción de estas enzimas durante la proteolisis post-mortem favorece la terneza de la carne (Ouali et al., 2006).

Los péptidos resultantes de la acción de las endopeptidasas son degradados por la acción de las exopeptidasas (aminopeptidasas, carboxipeptidasas, dipeptidasas y tripeptidasas) que hidrolizan las cadenas peptídicas a partir de sus extremos (Toldrá y Flores, 1998).

La concentración de sal influye en los procesos proteolíticos. En el jamón de cerdo blanco, las enzimas tienen mayor actividad cuando la concentración de sal es baja. La actividad enzimática va disminuyendo durante las sucesivas etapas del proceso, debido al aumento de la concentración de sal y a la pérdida de humedad. Sin embargo, en jamón Ibérico no se encontraron variaciones importantes de la proteolisis en un intervalo de concentración de sal entre 1,5 y 6,0 % (Córdoba et al., 1994).

La degradación de las proteínas también depende de la temperatura. Un aumento de la temperatura da lugar a una mayor proteolisis al aumentar la actividad de las enzimas proteolíticas. El efecto de la temperatura sobre la proteolisis ha sido estudiado en jamones Ibéricos con contenidos de sal similares (Martín et al., 1998). Igualmente, en jamones Serranos madurados con diferentes perfiles de temperaturas se ha comprobado el efecto de la integral térmica (sumatorio de temperaturas x tiempos de maduración) a lo largo del proceso de maduración sobre los productos resultantes de la proteolisis (péptidos de bajo peso molecular y aminoácidos libres), así como sobre la calidad y la intensidad del sabor (del Olmo et al., 2015).

Durante la maduración del jamón hay que tener también en cuenta la acción proteolítica de los microorganismos, fundamentalmente de aquellos presentes en la superficie del producto. Se ha encontrado actividad proteolítica en micrococáceas, levaduras y mohos aislados de jamón Ibérico (Rodríguez et al., 1998).

Los productos finales de la proteolisis son los aminoácidos libres, que están implicados en el sabor y aroma del jamón curado directa e indirectamente. Por una parte, algunos aminoácidos tienen un sabor característico y, si están en concentraciones superiores a su umbral de percepción, pueden contribuir al sabor del jamón. Por otra, los aminoácidos libres pueden sufrir reacciones como las de Maillard o la de Strecker, dando lugar a la formación de diversos compuestos volátiles (Ventanas et al., 1992), como se verá más adelante.

1.2.2. Lipolisis

La lipolisis afecta tanto a la fracción lipídica del músculo (grasa intramuscular) como a los lípidos del tejido adiposo (capa externa de grasa visible). La fracción lipídica del músculo está constituida fundamentalmente por triglicéridos y fosfolípidos, que son los sustratos naturales de las lipasas (ácida y neutra) y las fosfolipasas musculares (A1, A2, C y D). Estas enzimas hidrolizan los triglicéridos y los fosfolípidos generando ácidos grasos libres saturados, monoinsaturados y poliinsaturados (Motilva et al., 1993). En el músculo existen también una esterasa ácida, con un pH óptimo de 5,0 y una temperatura óptima de 30-45 ºC, y una esterasa neutra, con un pH óptimo de 7,5 y una temperatura óptima de 15-45 ºC. En el tejido adiposo hay también una esterasa ácida y una neutra con temperaturas óptimas de 60 ºC y 45-75 ºC, respectivamente (Motilva et al., 1992). Las enzimas lipolíticas muestran una gran estabilidad durante la maduración, aunque la liberación de ácidos grasos se produce fundamentalmente durante los primeros seis meses del proceso (Motilva y Toldrá, 1993). En una segunda etapa se generan compuestos volátiles como resultado de la oxidación química y/o enzimática de los ácidos grasos libres, que contribuyen al aroma del jamón curado.

Los lípidos del tejido adiposo están constituidos esencialmente (en un 99 %) por triglicéridos. La lipolisis en el tejido adiposo es debida a la acción de una lipasa sensible a hormona, que genera monoglicéridos y diglicéridos junto con gran cantidad de ácidos grasos libres (Toldrá y Flores, 1998). De manera simultánea y/o consecutiva a las reacciones enzimáticas de lipolisis, se dan también un

conjunto de reacciones químicas (autooxidación), tanto primarias (formación de peróxidos) como secundarias (formación de compuestos volátiles que contribuyen al aroma).

La oxidación, enzimática o no enzimática, de los ácidos grasos insaturados es un fenómeno complejo que tiene lugar en presencia de oxígeno, inducido por la luz, el calor, fotosensibilizadores y metales como el hierro hemímico y no hemínico. La oxidación no enzimática puede ocurrir por autooxidación y fotooxidación, dos mecanismos que requieren oxígeno, en distinta forma molecular. La autooxidación tiene lugar en presencia de $^{3}O_2$ y la fotooxidación en presencia de $^{1}O_2$. La oxidación enzimática, por la acción de la lipooxigenasa, difiere del proceso oxidativo no-enzimático y no es preponderante en carne y productos cárnicos (Mariutti y Bragagnolo, 2017).

La oxidación consta de tres fases (Estévez et al., 2009). En una primera fase (de iniciación) se forman radicales libres, por acción de la luz, los iones metálicos y el calor o por las actividades de las enzimas cicloxigenasa y lipoxigenasa. En la segunda fase (de propagación) se forman los hidroperóxidos, compuestos muy inestables y reactivos, que no tienen una contribución apreciable al aroma, pero que van a ser el origen de los productos secundarios de la oxidación. En la tercera fase (de terminación), los hidroperóxidos se escinden en moléculas volátiles de bajo peso molecular, principalmente aldehídos, aunque también pueden formarse cetonas, alcoholes, hidrocarburos y ácidos. Los hidroperóxidos también pueden reaccionar con proteínas, péptidos o aminoácidos y polimerizarse (Buscailhon et al., 1993) dando lugar a compuestos importantes en el aroma del producto final.

1.2.3. Reacciones de Maillard

Además de los compuestos procedentes de la degradación de proteínas y lípidos, otra fuente importante de compuestos responsables del aroma y sabor del jamón curado son las reacciones de Maillard, que se producen entre aminoácidos y grupos reductores como azúcares y otros compuestos carbonílicos, particularmente productos procedentes de la peroxidación lipídica (Antequera y Martín,

2001**)**. La fuente de nitrógeno necesaria para que se produzca esta reacción la proporcionan los aminoácidos libres, con sus grupos amino. Los aminoácidos básicos y con grupos hidroxilo reaccionan fuertemente con los dicarbonilos, mientras que los aminoácidos ácidos y los no polares tienen una menor actividad. Las reacciones de Maillard o de pardeamiento no enzimático tienen lugar normalmente durante el tratamiento térmico de los alimentos o bien durante almacenamientos prolongados a temperatura ambiente. Las condiciones durante la fase final de la maduración del jamón (temperatura entre 15 y 25 ºC, pH próximo a 6, niveles de a_w en torno a 0,85) así como el largo periodo de maduración favorecen la formación de este tipo de compuestos. La importancia de estas reacciones depende de las condiciones del procesado, existiendo una correlación entre el aumento de los productos derivados de las reacciones de Maillard y la temperatura y el tiempo de almacenamiento (Ventanas et al., 1992).

En las reacciones de Maillard hay cuatro fases consecutivas, la última de las cuales es la degradación de Strecker, que consiste en la desaminación oxidativa y la posterior descarboxilación de los aminoácidos en presencia de determinados compuestos como azúcares reductores o productos de oxidación lipídica (Arnoldi et al., 1987). En ella se forman los denominados aldehídos de Strecker, que son compuestos de bajo peso molecular con características aromáticas intensas que contribuyen al desarrollo del aroma final característico del jamón curado. En jamón Ibérico se ha descrito la existencia de aldehídos ramificados como 2-metilbutanal y 3-metilbutanal, cíclicos como feniletanal y azufrados como metiltiopropanal, así como sus alcoholes homólogos (2-metilbutanol, 3-metilbutanol, feniletanol y metiltiopropanol) cuya vía más probable de formación es la degradación de Strecker (García et al., 1991**)**. La gran cantidad de aminoácidos libres generados durante la maduración del jamón unido a la autooxidación, la baja a_w y el aumento de la temperatura favorece la formación de estos compuestos. Algunos aldehídos como el 3-metilbutanal pueden generarse también a través del metabolismo secundario de determinados microorganismos (Hinrichsen y Andersen, 1994).

1.3. Las altas presiones hidrostáticas y su aplicación en carne y productos cárnicos

1.3.1. Las altas presiones hidrostáticas

Con el objetivo de satisfacer la creciente demanda por parte de los consumidores de alimentos más frescos, con menos aditivos y más seguros desde el punto de vista microbiológico, la industria alimentaria desarrolla nuevas tecnologías de procesado y conservación. El tratamiento por altas presiones hidrostáticas (APH), clasificado como una pasteurización no térmica y de procesado mínimo, es una de las tecnologías emergentes para el procesado de alimentos que más ha crecido en los últimos años (Bajovic et al., 2012). A diferencia de otras técnicas de conservación de alimentos, las APH generan una percepción positiva por parte del consumidor (Cardello et al., 2007) y constituyen una herramienta muy útil para las empresas exportadoras de productos cárnicos envasados. Su principal objetivo es la inactivación de microorganismos y enzimas para aumentar la seguridad y la estabilidad del alimento, aunque esta técnica puede ser utilizada también para el desarrollo de productos alimentarios con nuevas texturas y nuevas características sensoriales (Serra et al., 2007a).

El fundamento de las APH consiste en someter los alimentos envasados a una presión hidrostática, empleando un fluido transmisor (Norton y Sun, 2008). La tecnología de APH se basa en dos principios físicos:

1) La ley de la isostática, según la cual la presión aplicada a los alimentos inmersos en un fluido, envasados o en contacto directo con dicho fluido, se transmite de forma instantánea y uniforme a todos los puntos del alimento, independientemente de sus dimensiones, forma y composición.

2) El principio de Le Châtelier, según el cual si un sistema en equilibrio sufre una perturbación el sistema responde al objeto de minimizar dicha perturbación. Por ello, las APH favorecen a aquellos fenómenos que vayan acompañados de una reducción de volumen y perjudican a los fenómenos que impliquen un aumento de volumen.

Los equipos industriales de APH suelen constar de una cámara de presurización, un sistema generador de presión (compuesto principalmente por un conjunto de bombas, un fluido poco

compresible transmisor de la presión que generalmente es agua y un sistema de válvulas) y un dispositivo de control de la temperatura (San Martín et al., 2002). El alimento, una vez envasado herméticamente en un material flexible, se introduce en la cámara de presurización. Esta se cierra, se llena con el fluido transmisor y se aumenta la presión hasta alcanzar el nivel deseado. Después, se detiene el bombeo de fluido y se cierran las válvulas, manteniéndose la presión constante durante el tiempo de tratamiento deseado. Los tratamientos empleados en la industria alimentaria varían entre 400 y 600 MPa durante 10 a 30 min, a temperaturas de 5 a 90 ºC (Garriga et al., 2002). Actualmente las condiciones más frecuentes en la industria cárnica son de 600 MPa durante 5 - 10 min a 10 - 25 ºC. Para poder mantener el alimento bajo presión durante un tiempo prolongado no es necesario un aporte extra de energía, lo que hace que el coste energético del tratamiento sea relativamente bajo (Cheftel, 1995).

A continuación se recogen algunos de los efectos que provocan las APH sobre los componentes de los alimentos en general y del jamón en particular.

La presión provoca modificaciones reversibles en algunas propiedades fisicoquímicas del agua cuando se aplica una compresión adiabática. Se produce, por ejemplo, una reducción de su volumen o un aumento ligero aumento de su temperatura (Cheftel, 1995). También se favorece la disociación del agua en ácidos débiles, produciendo una disminución del pH, y afecta a las fases de transición del agua, disminuyendo tanto el punto de fusión como el de congelación (Cheftel y Culioli, 1997).

En las proteínas la presión induce la ruptura de las interacciones relativamente débiles, como son las interacciones hidrofóbicas y electrostáticas. Sin embargo, los enlaces de hidrógeno pueden verse reforzados debido a la disminución de volumen que produce la presión. Los enlaces covalentes prácticamente no se ven afectados (Cheftel y Culioli, 1997; Bajovic et al., 2012). Las estructuras que estabilizan las proteínas presentan diferente sensibilidad a la presión, siendo las más sensibles la terciaria y cuaternaria. Los cambios en la estructura terciaria son producidos por presiones superiores a los 200 MPa. Por otro lado, la estructura secundaria solo se ve afectada por tratamientos a presiones

superiores a los 700 MPa, que producen cambios de estructura irreversibles (Rastogi et al., 2007; Bajovic et al., 2012). Debido a la naturaleza proteica de las enzimas, los mecanismos por los que las presiones les afectan son similares a los descritos para las proteínas en general. Las altas presiones modifican la estructura terciaria y cuaternaria de las enzimas pudiendo alterar su funcionalidad, así como disminuir o incluso aumentar su actividad (Hendrickx et al., 1998). La presión también puede afectar a la interacción enzima-sustrato, en el mecanismo de reacción o en una etapa limitante de la reacción (Cheftel, 1995).

La temperatura de fusión de los lípidos aumenta reversiblemente, más de 10 ºC cada 100 MPa (Cheftel, 1995), lo que favorece su cristalización. Este fenómeno podría ser uno de los responsables de la inactivación microbiana (Cheftel y Culioli, 1997).

1.3.2. Efecto de las APH sobre la microbiota de la carne y productos cárnicos

El efecto letal del tratamiento de APH sobre los microorganismos es uno de los principales atractivos que tiene esta tecnología para su uso por la industria cárnica. Además, las APH consiguen la inactivación de microorganismos y enzimas sin alterar apenas el contenido nutricional y las características sensoriales de los alimentos (Hoover et al., 1989; Norton y Sun, 2008).

La inactivación de los microorganismos en alimentos por las APH ha sido extensamente estudiada. Los cambios producidos en las células microbianas tras la aplicación de este tratamiento incluyen alteraciones en la membrana y la pared celular, en la morfología de las células microbianas, en las actividades enzimáticas y en los mecanismos genéticos (Hoover et al., 1989; Smelt, 1998; Tewari et al., 1999).

La membrana celular parece ser el primer punto afectado por las APH (Hoover et al., 1989). Entre los efectos del tratamiento de APH destacan los cambios de fase en las bicapas lipídicas, dando como resultado la disminución de la fluidez o incluso la ruptura de la membrana celular (San Martín et al., 2002), el desorden funcional de las proteínas de la membrana y finalmente la fragmentación

irreversible de la bicapa lipídica (Kato y Hayashi, 1999). Se han descrito algunos cambios morfológicos tras el tratamiento de APH como compresión del gas de las vacuolas, formación de poros en la membrana nucleica, modificaciones en los núcleos y los orgánulos intracelulares, modificaciones de las proteínas del citoplasma y liberación de los constituyentes intracelulares.

Las APH favorecen las reacciones que conllevan un cambio de volumen y suelen retrasar o en algunas ocasiones incluso inhibir aquellas reacciones en las que el volumen aumenta. Los efectos letales de las APH sobre los microorganismos también se pueden atribuir a la inactivación de enzimas. La inactivación de enzimas se debe a la alteración intramolecular de las estructuras con cambios conformacionales en su centro activo. La resistencia de las enzimas al tratamiento de APH es muy variable. Algunas enzimas son capaces de soportar presiones superiores a 500 MPa mientras que otras son inactivadas a 200 MPa (Patterson, 2005).

Al contrario que las proteínas, los ácidos nucleicos son relativamente resistentes a las APH probablemente por el gran número de puentes de hidrógeno intramoleculares que contienen. Sin embargo, a pesar de su relativa resistencia a la presión, los procesos de replicación y transcripción (mediados por enzimas) podrían verse interrumpidos (Smelt, 1998). Algunos estudios hacen referencia a la condensación del material nuclear en bacterias como *Listeria monocytogenes* o *Salmonella thompson* tras la aplicación de un tratamiento de APH (Mackey et al., 1994).

El grado de inactivación microbiana depende de parámetros tales como el tipo de microorganismo y su morfología, su estado fisiológico, la presión, el tiempo y la temperatura aplicada durante el proceso, así como el pH y la composición del alimento.

- Tipos de microorganismos y morfología

La sensibilidad de los microorganismos a la presión podría ser ordenada, de menor a mayor, de la siguiente manera: mohos y levaduras, bacterias Gram-negativas, bacterias Gram-positivas, virus y esporas bacterianas (Garriga et al., 2004). Los mohos y las levaduras son inactivados a presiones entre 200 y 300 MPa mientras que las bacterias Gram-negativas son destruidas generalmente a presiones

entre 300 y 500 MPa. Entre las bacterias Gram-positivas merecen una especial mención las especies del género *Staphylococcus* que pueden llegar a sobrevivir tras la aplicación de 500 MPa durante 60 min (Earnshaw et al., 1995). Algunos patógenos de interés alimentario como *E. coli* O157:H7 presentan una elevada resistencia a la presión (Patterson et al., 1995). Las esporas microbianas pueden resistir presiones superiores a 1.000 MPa (Kalchayanand et al., 1998). Por lo que se refiere a la morfología, los cocos son por lo general más resistentes a la presión que los bacilos (Patterson, 2005).

- Estado fisiológico

Los microorganismos en fase de crecimiento son más sensibles al tratamiento de APH que los que se encuentran en fase estacionaria, debido a que las células en fase estacionaria poseen una membrana citoplasmática más robusta que les permite soportar mejor el tratamiento (Mañas y Mackey, 2004).

- Condiciones de aplicación del tratamiento

Las condiciones de presión, temperatura y tiempo de tratamiento así como la composición, la a_w y el pH del alimento son factores que pueden condicionar los efectos de las APH sobre la supervivencia de los microorganismos.

Un aumento de la presión o el tiempo de tratamiento suele causar una mayor destrucción bacteriana, aunque no de forma lineal. La aplicación de presiones moderadas disminuye el crecimiento de los microorganismos mientras que las presiones elevadas provocan su inactivación (Serra et al., 2007a). El tratamiento a temperatura ambiente suele provocar una menor inactivación bacteriana que los tratamientos a bajas y altas temperaturas (Yuste et al., 2001).

- Composición del alimento

La composición del medio influye de manera decisiva sobre el efecto de las APH en los microorganismos. Los medios más ricos en nutrientes suelen aumentar la tolerancia de los microrganismos a las APH (Hoover et al., 1989). Una menor a_w está asociada a un menor crecimiento microbiano pero la resistencia de los microrganismos a las APH es mayor a niveles bajos de a_w (Garriga

et al., 2004). Los valores ácidos de pH aumentan la sensibilidad de los microorganismos a las APH (Smelt, 1998).

En la Tabla 1 se recogen algunos resultados relativos al efecto de las APH sobre la supervivencia de diferentes microorganismos en carne y algunos productos cárnicos.

1.3.3. Efecto de las APH sobre las características de la carne y los productos cárnicos

El tratamiento de la carne y los productos cárnicos por APH puede afectar a algunas de sus características relacionadas con la calidad organoléptica, tales como la textura, el color y el nivel de oxidación lipídica.

- Textura

El efecto de las APH sobre la textura de la carne y los productos cárnicos es un fenómeno complejo y en ocasiones de difícil interpretación. La presión y la temperatura a la que se aplican las APH son dos factores importantes a tener en cuenta. Se ha observado un aumento de la terneza de la carne de ternera con tratamientos de 200 MPa y temperaturas de 60-70 ºC, probablemente debido a un aumento de la proteolisis, mientras que al aplicar presiones de 200-800 MPa a temperaturas entre 20-40 ºC se registraba un aumento de la dureza **(**Ma y Ledward, 2004**)**. Además de la presión y la temperatura se debe tener en cuenta el estado en el que se encuentra la carne (pre- o post-*rigor mortis*) en el momento de aplicación de las APH.

En relación con la textura de la carne, algunos autores han observado una disminución de la actividad de enzimas proteolíticas tales como catepsinas y calpaínas tras la aplicación de APH (Ohmori et al., 1992; Cheftel y Culioli, 1997). Sin embargo, en otros estudios se ha registrado un aumento de su actividad, concretamente de la catepsina D en carne de ternera (Jung et al., 2000).

Tabla 1. Efecto de las APH sobre diferentes microorganismos en carne y productos cárnicos

Producto **Condiciones de procesado**	**Efecto sobre los microorganismos** **(Referencia)**
Carne de cerdo 400 MPa, 10 min, 25 ºC	*E. coli, C. jejuni, P. aeruginosa, S. typhimurium, Y. enterocolitica, S. cerevisiae, C. utilis* : reducción de 6 unidades logarítmicas (Shigehisa et al., 1991)
Carne de cerdo 300, 400 MPa, 30, 60, 90 s	*Toxoplasma gondii* : inactivación (Lindsay et al., 2006)
Carne de pavo 500 MPa, 1 min, 40 ºC	*L. monocytogenes* : reducción de 3,8 unidades logarítmicas (Chen et al., 2007)
Carne de pollo 600 MPa, 15 min, 20 ºC	*E. coli* O157:H7, *S. aureus* : reducción de 3 unidades logarítmicas (Patterson et al., 1995)
Carne de pollo 375 MPa, 10 min, 25 ºC	*C. jejuni* : reducción de 6 unidades logarítmicas (Solomon y Hoover, 2004)
Carne de pollo 300, 450, 600 MPa, 5 min, 15 ºC	*E. coli, S. typhimurium* : inactivación ; *L. monocytogenes* : niveles no detectables (Kruk et al., 2011)
Carne de ternera 200 MPa, 20 min, 20 ºC	*P. fluorescens* : reducción de 5 unidades logarítmicas (Carlez et al., 1993)
Carne de ovino 520 MPa, 4,2 min, 10 ºC	Viables totales : reducción de 2,5 unidades logarítmicas (Jung et al., 2003)
Ternera marinada 600 MPa, 6 min, 31 ºC	*S. aureus* : reducción de 2,67 unidades logarítmicas; bacterias lácticas : reducción de 3,99 unidades logarítmicas (Hugas et al., 2002)
Ternera marinada, jamón cocido y curado 600 MPa, 6 min, 31 ºC	*L. monocytogenes, S. enterica, S. aureus, Y. enterocolitica* y *D. hansenii* : inactivación (Jofré et al., 2009)
Jamón cocido 400, 600 MPa, 10 min, 22 ºC	*L. sakei, L. curvatus* : inactivación; *W. viridescens* y *L. mesenteroides* : supervivencia a 600 MPa (Han et al., 2011)
Jamón cocido 600 MPa, 6 min, 31 ºC	*S. aureus* : reducción de 1,12 unidades logarítmicas; bacterias lácticas : reducción de 4,57 unidades logarítmicas (Hugas et al., 2002)
Jamón cocido 400 MPa, 10 min, 17°C	*L. monocytogenes* : reducción de 1,9 unidades logarítmicas (Aymerich et al., 2005)
Jamón Serrano e Ibérico 450 MPa, 10 min, 12 ºC	*L. monocytogenes* Scott A : reducción de 1,16 - 1,50 unidades logarítmicas (Morales et al., 2006)
Jamón Serrano 400, 500, 600 MPa, 5 min, 12 ºC	*Salmonella* Enteritidis : reducción de 1,06 - 4,32 unidades logarítmicas (de Alba et al., 2012)
Jamón Serrano 400, 500 MPa, 10 min, 12 ºC	*E. coli* O157:H7 : reducción de 0,25 - 1,28 unidades logarítmicas (de Alba et al., 2013)

Además, tras la aplicación de APH se han observado cambios en las estructuras de los músculos tales como aumento de los espacios interfibrilares e intrafibrilares, modificaciones irreversibles en la estructura del sarcómero, hinchazón del retículo sarcoplásmatico, despolimerización de actina y miosina y disociación de la actomiosina (Cheftel y Culioli, 1997). Tratamientos a presiones superiores a 300-400 MPa causaban una fuerte desnaturalización de las proteínas miofibrilares y sarcoplásmicas y la conversión de la mioglobina / oximioglobina a la forma férrica desnaturalizada (Cheah y Ledward, 1996**)**.

- Color

El tratamiento de APH modifica de forma apreciable el color de la carne cruda, que puede llegar a adquirir un aspecto de carne cocida. La desnaturalización de la globina y/o el desplazamiento o liberación del átomo de hierro a 200-350 MPa, así como la oxidación de la mioglobina (Fe^{+2}) a metamioglobina (Fe^{+3}) a presiones de 400 MPa podrían explicar este cambio de color (Cheftel y Culioli, 1997). Sin embargo, las APH no producen cambios significativos en el color de los productos cárnicos cocidos, ya que las proteínas ya han sido desnaturalizadas durante el tratamiento térmico (Serra et al., 2007b). Los productos cárnicos curados y/o fermentados son por lo general bastante resistentes al tratamiento de APH, debido a que durante su proceso de elaboración la mioglobina se ha transformado en nitrosomioglobina, un pigmento muy resistente a la oxidación (Cheftel y Culioli, 1997).

- Oxidación lipídica

A partir de un determinado nivel de presión, el tratamiento de APH parece favorecer las reacciones de oxidación lipídica dependiendo de la temperatura y el nivel de oxígeno en contacto con el alimento. La carne de cerdo picada tratada a 300 MPa no mostraba oxidación lipídica. Sin embargo, la oxidación lipídica aumentaba al aumentar el nivel de presión a presiones superiores a 300 MPa, hasta los 800 MPa (Cheah y Ledward, 1996**).** Resultados similares se encontraron tras el

tratamiento de carne de pollo a 500 MPa (Beltrán et al., 2004). La presión a partir de la cual se favorece la oxidación lipídica varía de unas carnes a otras. La carne de ternera parece ser más susceptible que otras carnes, mostrando signos de oxidación lipídica con presiones de 200 MPa (Ma et al., 2007), mientras que la carne de pollo los muestra tan solo a partir de 500 MPa (Orlien et al., 2000; Beltrán et al., 2003).

La presurización no parece aumentar la oxidación lipídica en los productos cárnicos. El tratamiento de salchichón por APH (500 MPa, 5 min, 18 ºC) no conllevó un incremento de la oxidación lipídica (Rubio et al., 2007b). Tampoco se observó un aumento de la oxidación lipídica en fuet y chorizo tratados por APH (400 MPa, 10 min, 17 ºC), tal vez debido a que este tipo de reacciones ya habían tenido lugar durante la maduración de dichos embutidos (Marcos et al., 2007).

1.4. El efecto de las APH sobre el jamón curado

Se han llevado a cabo diversos estudios sobre el efecto de las APH en la microbiología, el color, la textura, las actividades enzimáticas, la oxidación lipídica, los compuestos químicos y las características sensoriales del jamón curado.

1.4.1. Efecto de las APH sobre la microbiota del jamón curado

Garriga et al. (2004) observaron que en jamón curado loncheado, envasado al vacío y tratado a 600 MPa durante 6 min a 31 ºC, la microbiota total disminuía al menos 2 unidades logarítmicas con el tratamiento y se mantenía en valores bajos (alrededor de 3 log ufc / g) durante 120 días de almacenamiento en refrigeración. Morales et al. (2006) investigaron la inactivación de *L. monocytogenes* en jamón loncheado envasado al vacío y tratado a 450 MPa durante 10 min a 12 ºC, registrando descensos de 1,16 y 1,50 log ufc / g en jamón Serrano y jamón Ibérico, respectivamente, durante el tratamiento por APH y de 2,09 y 0,89 log ufc / g, respectivamente, durante la primera

semana de almacenamiento en refrigeración. Atribuyeron la mayor supervivencia del patógeno durante el tratamiento en jamón Serrano a su mayor contenido de sal y su menor a_w y la mayor supervivencia durante el almacenamiento en jamón Ibérico a su mayor contenido de grasa. Clariana et al. (2011) comprobaron que en jamón curado loncheado, envasado al vacío y tratado a 600 MPa durante 6 min a 15 ºC, se producía una disminución significativa en los niveles de microorganismos aerobios totales a lo largo de los 50 días de almacenamiento en refrigeración, con unos niveles de enterobacteriáceas por debajo de los límites de detección. De Alba et al. (2012) observaron disminuciones de *Salmonella* Enteritidis en jamón Serrano tratado a 400, 500 y 600 MPa durante 5 min a 12 ºC de 1,06, 2,54 y 4,32 unidades logarítmicas, respectivamente. En un estudio similar, de Alba et al. (2013) registraron descensos de los niveles de *E. coli* O157:H7 en jamón Serrano tratado a 400 y 500 MPa durante 10 min a 12 ºC de 0,25 y 1,28 unidades logarítmicas, respectivamente. Bover-Cid et al. (2015) estudiaron la influencia de la a_w (0.86 - 0.96) y el contenido de grasa (10 - 50 %) sobre la reducción de *L. monocytogenes* en jamón curado tras la aplicación de APH (347 - 852 MPa, 5 min, 15 ºC), observando que valores bajos de a_w tenían un efecto protector a cualquier nivel de presión y que el alto contenido de grasa tenía un efecto protector por encima de 700 MPa, mientras que a menor presión los contenidos de grasa superiores al 30 % favorecían la inactivación de *L. monocytogenes*. Trabajando en las mismas condiciones, Bover-Cid et al. (2017) comprobaron que con una a_w de 0,88 la reducción de los niveles de *Salmonella enterica* apenas variaba al aumentar la presión (2,3 log ufc / g a 450 MPa y 3,2 log ufc / g a 750 MPa) mientras que con una a_w de 0,96 la reducción de los niveles de *S. enterica* aumentaba considerablemente al aumentar la presión (3,3 log ufc / g a 450 MPa y 8,9 log ufc / g a 750 MPa). No se observó un efecto protector de la grasa sobre el patógeno.

1.4.2. Efecto de las APH sobre las características del jamón curado

- Textura

Por lo que respecta a la textura, Serra et al. (2007b) comprobaron que el tratamiento de jamón curado a 400 y 600 MPa causaba pequeñas variaciones en los parámetros de textura mientras que Clariana et al. (2011) observaron que tras un tratamiento del jamón curado a 600 MPa durante 6 min a 15-32 ºC aumentaban la dureza y la fuerza de masticación. Por su parte, de Alba et al. (2012) registraron valores más bajos de los parámetros de textura en muestras de jamón Serrano tratadas a 500 y 600 MPa que en las muestras control y en las tratadas a 400 MPa, siendo mayor el efecto del tiempo de almacenamiento que el efecto del tratamiento por APH.

- Color

El color del jamón curado es una de las características organolépticas más apreciadas por los consumidores. Durante la maduración del jamón cambian sus parámetros colorimétricos, disminuyendo la luminosidad (L*) e incrementándose la intensidad del color rojo (a*) (del Olmo et al., 2013). Algunos autores (Andrés et al., 2004, 2006) han observado que tras el tratamiento del jamón curado por APH se producían incrementos del parámetro L* y descensos del parámetro a*. En jamón Serrano e Ibérico tratados a 450 MPa durante 10 min a 12 ºC no se registraron variaciones en los parámetros L* y a* mientras que el parámetro b* aumentaba, pero solamente en jamón Ibérico (Morales et al., 2006). Los diferentes efectos encontrados en el color del jamón curado tras el tratamiento de APH se han atribuido a diferencias iniciales en la composición del pigmento del jamón, aunque las variaciones de los parámetros de color respecto del control después de tratamientos a 400 y 600 MPa eran pequeñas (Serra et al., 2007b). En otro estudio no se encontraron pérdidas de color al tratar el jamón Ibérico a presiones entre 200 y 300 MPa durante 15 y 30 min (Cava et al., 2009). Un tratamiento de APH a 600 MPa ocasionaba un aumento del parámetro L* del jamón curado y una reducción de la intensidad del color (Clariana et al., 2011). En jamón Serrano

tratado a 400, 500 y 600 MPa se observaron valores de L* más elevados y valores de a* más bajos en las muestras tratadas a 500 y 600 MPa que en el resto, mientras que los valores de b* eran más bajos en las muestras tratadas a 600 MPa que en el resto.

- Oxidación lipídica

La oxidación lipídica durante la maduración del jamón curado contribuye al desarrollo de las características de olor, aroma y sabor del producto (Toldrá y Flores, 1998). El efecto de las APH sobre la oxidación lipídica es un aspecto muy importante que se debe valorar cuando se procesa un producto cárnico como el jamón curado.

La actividad de las enzimas antioxidantes (catalasa, glutatión peroxidasa y superóxido dismutasa) disminuye ligeramente tras el tratamiento por APH en jamón que se encuentra en etapas iniciales de su proceso de elaboración (Serra et al., 2007a). Sin embargo, no se observa un efecto del tratamiento de APH sobre las actividades enzimáticas antioxidantes en jamón curado (Clariana et al., 2011).

En jamón Ibérico loncheado, las APH tuvieron un efecto reducido sobre la oxidación lipídica a presiones inferiores a 300 MPa pero el efecto aumentaba a presiones superiores (Andrés et al., 2004, 2006). Al tratar jamón Ibérico loncheado a 200 y 300 MPa durante 15 o 30 min se observó un aumento de la oxidación lipídica al aumentar la presión y el tiempo de tratamiento, aunque tras 90 días de almacenamiento en refrigeración las diferencias entre las muestras control y las tratadas por APH no fueron significativas (Cava et al., 2009). Al aplicar un tratamiento de 600 MPa durante 6 min a 12 ºC en jamón Ibérico, seguido de almacenamiento en refrigeración durante 30 días con ciclos luz / oscuridad de 12 h, se observó un aumento significativo en los niveles de oxidación de lípidos y proteínas (Fuentes et al., 2010). En jamón Serrano loncheado tratado a 600 MPa durante 6 min a 15-32 ºC se mantuvo la estabilidad oxidativa de los lípidos (Clariana et al., 2011). Sin

embargo, en muestras de jamón Serrano tratadas a 400, 500 y 600 MPa los niveles de oxidación de lípidos aumentaron tras 30 y 60 días de almacenamiento en refrigeración (de Alba et al., 2012).

- Compuestos volátiles

El efecto del tratamiento de APH sobre los compuestos volátiles del jamón curado ha sido objeto de varios estudios. En jamón Serrano, Rivas-Cañedo et al. (2009a) investigaron el efecto de un tratamiento a 400 MPa durante 10 min a 12 ºC, seguido de almacenamiento en refrigeración durante 3 días, comprobando que la mayoría de los compuestos afectados por el tratamiento tenían niveles menores en las muestras tratadas por APH, a excepción de la 2-heptanona que incrementó su nivel tras el tratamiento. Estos autores no registraron un aumento en los niveles de los compuestos volátiles procedentes de reacciones de oxidación lipídica tras el tratamiento. Por el contrario, Fuentes et al. (2010) observaron tras el tratamiento de jamón Ibérico a 600 MPa durante 6 min a 12 ºC, seguido de almacenamiento en refrigeración durante 30 días, un aumento significativo en los niveles de aldehídos lineales.

El efecto del tratamiento de APH sobre los compuestos volátiles derivados de las reacciones de Maillard y la degradación de Strecker es variable. Así, al tratar jamón Serrano a 400 MPa, Rivas-Cañedo et al. (2009a) no observaron efectos significativos sobre ninguno de los compuestos volátiles procedentes de estas rutas. Por el contrario, al tratar jamón Ibérico a 600 MPa, Fuentes et al. (2010) registraron una disminución de los niveles de 2-metilbutanal y 3-metilbutanal en el formato de envase de loncheado convencional, un aumento de pentanal en el formato de loncheado apilado en vertical, un aumento de heptanal en el loncheado convencional, y un aumento de hexanal en los dos formatos de loncheado.

- Características sensoriales

El tratamiento de APH no parece tener una gran influencia sobre las características de olor y sabor del jamón curado. Así, Morales et al. (2006) no encontraron diferencias significativas en el

sabor de jamón Serrano y jamón Ibérico después de un tratamiento a 450 MPa durante 10 min a 12 ºC. Igualmente, Clariana et al. (2011) encontraron que en jamón tipo Serrano loncheado tratado a 600 MPa la intensidad del olor disminuía inmediatamente después del tratamiento pero que al cabo de 50 días el jamón tratado por APH mostraba una mayor intensidad de olor y una mayor retención de compuestos del aroma. Estos autores observaron que la percepción del sabor salado era mayor en el jamón tratado por APH, lo que fue atribuido a una mayor accesibilidad de los iones Na^+ en dichas muestras. Sin embargo, Fuentes et al. (2014) comprobaron que las muestras de jamón Ibérico tratadas a 600 MPa tenían una mayor intensidad de sabor y un sabor más salado y más rancio que las no tratadas, según los resultados de un análisis sensorial dinámico llevado a cabo aplicando el método tiempo-intensidad.

1.5. Objetivos

A la vista de los resultados obtenidos en estudios previos sobre los compuestos volátiles y la microbiota del jamón curado, el plan de trabajo de la presente Tesis se diseñó de acuerdo con los objetivos siguientes:

- Investigar la influencia de la composición química y las altas presiones hidrostáticas sobre los compuestos volátiles presentes en jamón Serrano, después de 3 días y de 5 meses de almacenamiento en refrigeración a 4 ºC.
- Estudiar el efecto de las altas presiones hidrostáticas sobre la microbiota del jamón Serrano con diferentes contenidos de grasa intramuscular y sal.
- Investigar la influencia de la composición química y las altas presiones hidrostáticas sobre los compuestos volátiles presentes en jamón Ibérico, después de 3 días y de 5 meses de almacenamiento en refrigeración a 4 ºC.
- Estudiar el efecto de las altas presiones hidrostáticas sobre la microbiota del jamón Ibérico con diferentes contenidos de grasa intramuscular y sal.

2. Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham

Artículo publicado en *Meat Science* 111 (2016) 130-138

Meat Science 111 (2016) 130–138

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham

Nerea Martínez-Onandi, Ana Rivas-Cañedo, Manuel Nuñez *, Antonia Picon

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, Madrid 28040, Spain

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 9 September 2015
Accepted 10 September 2015
Available online 14 September 2015

List of chemical compounds:
3-Hydroxy-2-butanone (PubChem CID: 179)
Ethanol (PubChem CID: 702)
Methanethiol (PubChem CID: 878)
2-Propanol (PubChem CID: 3776)
1-Pentanol (PubChem CID: 6276)
2-Methylbutanal (PubChem CID: 7284)
2-Butoxyethanol (PubChem CID: 8133)
Dimethyldisulfide (PubChem CID: 12232)
2-Pentanol (PubChem CID: 22386)
Ethyl 2-methyl butanoate (PubChem CID: 24020)

Keywords:
Serrano dry-cured ham
Volatile compounds
High pressure processing
Salt content
Intramuscular fat

ABSTRACT

The volatile fraction of 30 Serrano dry-cured hams with different salt and intramuscular fat contents was investigated. In addition, the effect of high pressure processing (HPP) at 600 MPa for 6 min at 21 °C on the volatile compounds of those hams was studied. One hundred volatile compounds were identified and their levels subjected to analysis of variance with ham chemical composition (a_w, salt content, intramuscular fat content and salt in lean ratio) and HPP treatment as main effects. Chemical composition mainly affected the relative abundance of acids, alcohols, branched-chain aldehydes, ketones, benzene compounds, sulfur compounds and some miscellaneous compounds. Salt content and fat content influenced a greater number of volatile compounds than a_w. High pressure processing had a significant effect on only 8 volatile compounds, with higher levels of methanethiol and sulfur dioxide in HPP-treated samples and higher levels of ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, dimethyl disulfide and dimethyl trisulfide in control untreated samples.

1. Introduction

Serrano ham is a traditional Spanish dry-cured meat product, highly appreciated worldwide. Approximately 18 million Serrano hams are annually produced. Its manufacturing process begins with a salting step, during which salt and other curing ingredients (nitrate and/or nitrite) and additives (ascorbic acid) slowly diffuse into the meat, followed by brushing or washing of hams to remove the excess of salt, a post-salting step and a ripening or drying stage. The whole process usually lasts from 7 to 12 months.

Aroma, one of the most important sensory characteristics of dry-cured ham, is associated with its volatile composition. The volatile fraction of Serrano ham is mainly composed of aldehydes, ketones, alcohols, hydrocarbons, lactones and esters (Flores, Grimm, Toldrá, & Spanier, 1997; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998; Toldrá & Flores, 1998). The presence of most of these volatile compounds in other dry-cured ham varieties has been reported (Barbieri et al., 1992; Berdagué, Denoyer, Le Quéré, & Semon, 1991; Carrapiso, Ventanas, & García, 2002; García et al., 1991; Luna, Aparicio, & García-González, 2006). The complex biochemical reactions that take place through the ripening period are responsible for the characteristic aroma and volatile composition of each of those dry-cured ham varieties. Proteolysis and lipolysis generate peptides, free amino acids and free fatty acids that contribute to the flavor and aroma of the product. These reactions are mainly due to endogenous enzymes, with minor contribution from enzymes of microbial origin. Lipid oxidation and further interaction of the resulting compounds with proteins, peptides and free amino acids, as well as Strecker degradation of free amino acids and Maillard reactions, are responsible for the generation of most of the volatile compounds found in dry-cured ham (Toldrá & Flores, 1998; Ventanas et al., 1992).

Salting of ham plays an important role in the manufacturing process, since NaCl contributes to microbial stability through the reduction of a_w, enhances protein solubilization, affects proteolysis, lipolysis and lipid

* Corresponding author.

http://dx.doi.org/10.1016/j.meatsci.2015.09.004
0309-1740/

oxidation, improves product texture and contributes directly to flavor (Toldrá & Flores, 1998). However, the relatively high NaCl and fat contents of Serrano ham fail to meet the consumers' demand for healthier food products with lower salt and fat contents. Salt content of ham may be reduced, but lower NaCl concentration increases microbial risk and may cause technological problems. Thus, dry-cured hams of low salt content showed more pronounced rancid, fatty and buttery aroma notes than hams of high salt content (Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Casabianca, 1999). Iberian ham of 6% salt content was drier, harder and more fibrous than ham of 3% salt content, but the effect of salt content on aroma traits was not significant (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004b) and the differences in salt content (3% or 6%) hardly influenced the levels of volatile compounds, except for 2-pentylfuran (Andres, Cava, Ventanas, Muriel, & Ruiz, 2007). A lower fat content would negatively affect the sensory characteristics of dry-cured ham, since lipids are the substrates for volatile compound formation through chemical reactions (Toldrá & Flores, 1998). Fat not only acts as a reservoir for certain volatile compounds coming from the diet (Ventanas, Estevez, Andrés, & Ruiz, 2008), but also affects taste perception of sweet, salty, sour and bitter stimuli (Lynch, Liu, Mela, & MacFie, 1993) and could influence volatile compound release to the mouth, by retaining in particular non-polar compounds (Ventanas et al., 2008). The influence of salt and fat contents on the perception of flavor and texture in dry-cured hams depends on the variety, being more marked for Iberian ham than for Serrano ham (Lorido, Estévez, Ventanas, & Ventanas, 2015).

High pressure processing (HPP) is a non-thermal technology with a minimal impact on the nutritional and sensory characteristics of meat and meat products (Cheftel & Culioli, 1997). For this reason, HPP is being widely used in the meat industry to eliminate pathogens and spoilage microorganisms, thus ensuring product safety and increasing its shelf-life (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004).

The effect of HPP on the volatile fraction of meat products greatly depends on treatment conditions, particularly the pressure level, and on the compositional characteristics of the product (Rivas-Cañedo, Juez-Ojeda, Nuñez, & Fernández-García, 2011). There is little information available on the effect of HPP on the volatile fraction of dry-cured ham. Lower levels of nonane, decane, undecane, 2-undecene, dodecane, ethyl pentanoate and benzaldehyde and higher levels of 2-heptanone were reported in HPP-treated Serrano ham, independently of the packaging material, while 17 volatile compounds increased and 19 compounds decreased after HPP, depending on the packaging material (Rivas-Cañedo, Fernández-García, & Nuñez, 2009a). Enhanced lipid oxidation and protein oxidation were observed in Iberian ham treated at 600 MPa, with a more pronounced effect on ham slices than on non-sliced samples (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010). Taking into account these results, interactions between HPP treatment and ham characteristics, including its chemical composition, are to be expected. However, the effect of HPP on the volatile compounds of Serrano dry-cured ham with different salt and fat contents has not been studied, to our knowledge.

The objective of the present work was to investigate the influence of Serrano ham chemical composition on the formation of volatile compounds during ripening and to elucidate the changes caused by HPP treatment in the volatile fraction of Serrano hams of different chemical compositions.

2. Materials and methods

2.1. Selection and manufacture of Serrano hams

Manufacture of Serrano hams was carried out at the Institute of Food and Agricultural Research and Technology (IRTA, Monells, Spain). Thirty green hams were selected at commercial slaughterhouses from animals of different genotypes in order to obtain a wide range of fat contents. Twenty-one hams were from Large White × Landrace animals and nine hams from animals with a minimum of 50% Duroc breed. Fat content of entire hams was determined using magnetic resonance sensor technology (Jmp Ingenieros, Sotés, Spain). Homogeneous hams in terms of weight and pH were used in this study. Average weight of hams was 11.77 kg (SD, 0.66 kg) whereas the pH in the semimembranosus muscle at 24 h post-mortem ranged from 5.4 to 5.9. Hams were manually rubbed with the following mixture (per kg of raw ham): 10 g NaCl, 1.0 g dextrose, 0.5 g ascorbic acid, 0.15 g KNO_3 and 0.15 g $NaNO_2$. Afterwards, hams were held with excess of salt at 3 ± 2 °C and 85 ± 5% RH. In order to obtain a wide range of salt and fat contents, 0.6 to 1.5 days of salting per kg of raw ham were applied and hams of different fat contents were selected for each of the salting times, which ranged from 7 to 15 days. After salting, hams were washed with cold water, weighed and hung in a cold room at 3 °C and 75–80% RH to rest. Temperature was progressively increased up to 20 °C during ripening. The process was finished when a total weight loss of 36% was achieved.

2.2. Sampling and high pressure processing

Two slices (approximately 150 g) from the cushion (mainly composed of the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* muscles) were obtained from each ham and individually vacuum-packaged. One of the slices was HPP-treated at 600 MPa for 6 min at 21 °C (pressure build up time, 2.5 min; pressure release time < 2 s) in a 120 L capacity Wave 6000 equipment (Hiperbaric, Burgos, Spain) at IRTA (Monells, Spain) whereas the other slice served as untreated control. Ham slices were held at 4 °C for 3 days and kept at −35 °C at our laboratory until analysis, which took place within 1 month of sampling.

2.3. Physicochemical determinations

Ham homogenates, approximately 50 g in weight and representative of the whole slice, were obtained using a mechanical grinder (IKA Labortechnik, Staufen, Germany). Chloride content was determined by the Volhard method (AOAC, 2000) and intramuscular fat content after extraction with chloroform–methanol (Folch, Lees, & Sloane-Stanley, 1957) on representative subsamples of the homogenate, approximately 3 g in weight. Water activity (a_w) was measured using an AquaLab Series 3-equipment (Decagon Devices, Inc., Pullman, WA, USA), according to the manufacturer instructions. Analyses were performed in triplicate.

2.4. Analysis of volatile compounds

Volatile compounds were extracted by solid-phase microextraction (SPME) and analyzed by gas chromatography–mass spectrometry (GC–MS) (HP 6890-MSD HP 5973, Agilent, Palo Alto, CA, USA). Fifteen grams of Serrano ham, trimmed of subcutaneous fat and representative of the whole ham slice, were homogenized in a mechanical grinder with 15 g of anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) and 30 μL of an aqueous solution of 534 mg/L cyclohexanone (Sigma-Aldrich, Alcobendas, Spain) added as internal standard. A 40 mL headspace glass vial was filled with 10 g of the mixture, sealed with a polytetrafluoroethylene (PTFE) faced silicone septum and introduced in a thermostatic bath at 35 °C (D3 model, Haake, Berlin, Germany) for both equilibration and extraction phases (1 h each). An SPME manual holder equipped with a 2 cm × 50/30 μm StableFlex Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) coated fiber (Supelco, Bellefonte, PA, USA) was inserted through the PTFE septum for headspace extraction. Desorption into the GC injection port took place at 260 °C for 10 min in splitless mode. Chromatographic separation was carried out in a Zebron 100% polyethylene glycol capillary column (60 m long; 0.25 mm internal diameter; 0.50 μm film thickness; ZB-WAXplus, Phenomenex, Torrance, CA, USA) with 1 mL/min helium flow, with the following temperature program: 16 min at 45 °C, a first ramp at 4 °C/min to 110 °C, 9 min at 110 °C, a second ramp at 15 °C/min to 230 °C and 3 min at 230 °C, a final ramp at 10 °C/min

to 250 °C and 2 min at 250 °C. Detection was performed with electron impact ionization, with 70 eV ionization energy operating in the full-scan mode at 1.74 scans/s from 33 to 280 amu. Interface, source and quadrupole temperatures were 280, 230 and 150 °C, respectively. Compound identification was carried out by injection of commercial standards, by spectra comparison using the Wiley7Nist05 Library (Wiley & Sons Inc., Germany), and/or by calculation of linear retention indices (LRI) relative to a series of alkanes (C5–C20). The sums of the abundances of selected characteristic ions were used for the semi-quantitative determination of volatile compounds. To ease comprehension by the reader, the data obtained were multiplied by 10^{-5}. Each ham was analyzed in triplicate.

2.5. Statistical analysis

Data were analyzed using the SPSS 12.0 statistical package (S.P.S.S. Inc., Chicago, IL, USA). Chromatographic areas were subjected to analysis of variance with HPP treatment or one of the compositional parameters a_w, salt content, fat content or salt in lean ratio, defined as salt / (100 − fat), as main effects (one-way ANOVA). Three groups of hams for low, medium and high values of each of the compositional parameters were set by using the mean ± 0.5 standard deviations (SD) as the criterion for the separation of hams into groups. On the basis of the area of the normal curve (Rohlf & Sokal, 1969), 38.30% of the samples would be included in the mean ± 0.5 SD mid-value group, 30.85% in the group of lower values and 30.85% in the group of higher values. Comparison of the levels of volatile compounds between the three groups of hams was performed by using Tukey's test, with the significance assigned at $P < 0.05$.

3. Results

One hundred compounds were detected in the volatile fraction of Serrano dry-cured ham by means of SPME followed by GC–MS. Table 1 lists the compounds grouped by chemical families, together with their linear retention indexes, the ions used for quantification and the methods used for identification. The volatile compounds found included 7 acids, 21 alcohols, 7 aldehydes, 6 alkanes, 5 esters, 10 ketones, 20 benzene compounds, 7 non-cyclic sulfur compounds, and 17 miscellaneous compounds, among them 6 furanes, 2 furanones, 3 pyrazines and 2 terpenes.

3.1. Effect of ham chemical composition

Water activity (a_w) of Serrano hams ranged from 0.833 to 0.883, with a mean value of 0.859 (SD, 0.013). They were grouped into 11 low a_w (<0.853) hams, 9 medium a_w (0.853–0.864) hams and 10 high a_w (>0.864) hams. a_w influenced significantly the levels of 19 volatile compounds in untreated control samples and of 18 volatile compounds in HPP-treated samples, while 13 compounds were affected significantly in untreated as well as in HPP-treated samples. High a_w hams had higher levels of ethanol, 2-propanol, 1-methoxy-2-propanol, ethyl acetate and dimethyl sulfide than low a_w hams while low a_w hams showed higher levels of propanoic and hexanoic acids, 3-ethyl cyclopentanone, 3-ethyl phenol, 3-phenoxy-1-propanol, 2-methylfuran, 2,5-dimethylfuran and 5-ethyl dihydro-2(3H)furanone (Table 2).

NaCl concentration of hams ranged from 2.87% to 7.91%, with a mean value of 5.49% (SD, 1.30%). They were grouped into 7 hams of low (<4.83%), 13 of medium (4.83–6.13%) and 10 of high (>6.13%) salt content. Salt concentration influenced significantly the levels of 28 volatile compounds in untreated control samples and of 35 volatile compounds in HPP-treated samples, while 23 compounds were affected significantly in untreated as well as in HPP-treated samples. High NaCl content hams had higher levels of 1-butanol, 1-pentanol, 1-octanol, 2-butanol, 2-pentanol, 1-penten-3-ol, 2-methyl-2-buten-1-ol, 2-butoxyethanol, 3-hydroxy-2-butanone, 4-methyl-2-pentanone, methylbenzene, 4-methylphenol, *p*-nitrophenyl hexanoate and pyridine than low NaCl content hams whereas low NaCl content hams showed higher levels of 2-methylbutanal, benzenemethanol, 3-phenoxy-1-propanol, methanethiol, dimethyl trisulfide, 2-methylfuran, methylpyrazine, 2,6-dimethylpyrazine and 2-methylthiazole (Table 3).

Salt-in-lean ratio of hams ranged from 0.033 to 0.081, with a mean value of 0.058 (SD, 0.013). They were grouped into 7 hams of low (<0.050), 13 of medium (0.050–0.066) and 10 of high (>0.066) salt-in-lean ratio. The salt-in-lean ratio influenced significantly 27 volatile compounds in untreated control samples and 32 volatile compounds in HPP-treated samples, while 18 compounds were affected significantly in untreated as well as in HPP-treated samples. High salt-in-lean ratio hams had higher levels of 1-butanol, 1-pentanol, 1-octanol, 1-penten-3-ol, 2-methyl-2-buten-1-ol, 2-butoxyethanol, 3-hydroxy-2-butanone, 4-methyl-2-pentanone, benzenemethanol, 4-methylphenol, *p*-nitrophenyl hexanoate and pyridine than low salt-in-lean ratio hams while low salt-in-lean ratio hams showed higher levels of 2-methylbutanal, methanethiol, dimethyl trisulfide, methylpyrazine, 2,6-dimethylpyrazine and 2-methylthiazole (Table 4).

Intramuscular fat content of hams ranged from 1.83% to 14.03%, with a mean value of 5.28% (SD, 3.45%). They were grouped into 8 hams of low (<3.56%), 11 of medium (3.56–6.99%) and 11 of high (>6.99%) intramuscular fat content. Fat content influenced significantly the levels of 37 volatile compounds in untreated control samples and of 42 volatile compounds in HPP-treated samples, while 28 compounds were affected significantly in untreated as well as in HPP-treated samples. High fat content hams had higher levels of propanoic, hexanoic and octanoic acids, 2-methylbutanal, 2,3-pentanedione, phenol, 3-ethyl-phenol, 3-phenoxy-1-propanol, dimethyl trisulfide, 2-methylfuran, 2,5-dimethylfuran, 2,6-dimethylpyrazine and 2-methylthiazole than low fat content hams whereas low fat content hams showed higher levels of 1-butanol, 1-pentanol, 2-propanol, 2-butanol, 2-pentanol, 2-methyl-2-buten-1-ol, 2-butoxyethanol, 1-methoxy-2-propanol, 2-(2-ethoxyethoxy)-ethanol, 4-methyl-2-pentanone, benzonitrile, 2-phenoxy-ethanol, 4-methyl-phenol, naphthalene and pyridine (Table 5).

3.2. Effect of high pressure processing

Only 4 esters and 4 non-cyclic sulfur compounds were significantly influenced by HPP treatment of Serrano ham, out of the 100 volatile compounds found (Table 6). The levels of ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, dimethyl disulfide and dimethyl trisulfide were significantly higher in untreated samples while the levels of methanethiol and sulfur dioxide were significantly higher in HPP-treated samples.

4. Discussion

The 100 volatile compounds identified in the present work, after SPME of Serrano ham samples, is a slightly higher number than the 84 to 93 compounds identified in Serrano ham after sample extraction by purge-and-trap dynamic headspace in previous studies (Flores et al., 1997; Rivas-Cañedo et al., 2009a; Sabio et al., 1998) and higher than the 55 compounds found in SPME samples of dry-cured ham (Ramírez & Cava, 2007), although lower than the more than 150 volatile compounds reported for SPME samples of dry-cured ham inoculated with selected fungal strains (Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006). Seven carboxylic acids were found in the present study while Sabio et al. (1998) and Rivas-Cañedo et al. (2009a) did not detect any, Flores et al. (1997) only detected acetic acid and Martín et al. (2006) detected 12 carboxylic acids. In contrast, branched-chain alkanes, found by Flores et al. (1997), Martín et al. (2006) and Rivas-Cañedo et al. (2009a), were not detected by Sabio et al. (1998) or in the present study. The origin of branched-chain alkanes, considered to be non-contributors to meat flavor (Shahidi, Rubin, & Dsouza, 1986), has been traced back to

Table 1
Volatile compounds identified in untreated and HPP-treated samples of Serrano ham.

Compound	LRI[a]	QI[b]	ID[c]
Acids			
Acetic acid	1469	43,45,60	MS
Propanoic acid	1549	45,57,74	ST, MS
Butanoic acid	1641	60,73,88	ST, MS
Pentanoic acid	1682	43,60,87	ST, MS
Hexanoic acid	1855	41,60,87	ST, MS
Octanoic acid	2063	43,73,101,115	ST, MS
2-Methyl propanoic acid	1578	43,73,88	ST, MS
Alcohols			
Ethanol	941	45,46,43,41	MS
1-Butanol	1165	56,41,43,42	ST, MS
1-Pentanol	1265	42,55,41,70	ST, MS
1-Hexanol	1364	56,55,69,41	ST, MS
1-Heptanol	1479	70,56,55,69	ST, MS
1-Octanol	1582	56,55,69,70	ST, MS
2-Propanol	934	45,43,41,59	MS
2-Butanol	1046	45,59	ST, MS
2-Pentanol	1141	45,73	ST, MS
2-Hexanol	1236	45,43,69,57	ST, MS
2-Heptanol	1329	45,55,43,41	MS
2-Methyl-1-propanol	1112	42,43,41,74	ST, MS
3-Methyl-1-butanol	1225	55,70,42,43	ST, MS
1-Penten-3-ol	1177	57,41,43,88	MS
1-Octen-3-ol	1471	57,72,55	ST, MS
2-Methyl-2-buten-1-ol	1333	53,71,86	MS
2-Methyl-3-buten-2-ol	1060	53,59,65,71	MS
2-Butoxyethanol	1431	57,75,87,100,108	MS
2-(2-Ethoxyethoxy)-ethanol	1653	45,59,72,104	MS
1-Methoxy-2-propanol	1148	45,59,75,90	MS
4-Butoxy-1-butanol	1724	57,71,103,113,129	MS
Aldehydes			
Hexanal	1097	56,57,72	ST, MS
Heptanal	1199	70,44,43,55	ST, MS
Nonanal	1413	57,98,70,82	ST, MS
Dodecanal	1739	57,82,68,96	ST, MS
2-Methylpropanal	816	41,43,72	MS
2-Methylbutanal	917	57,41,58,39	ST, MS
3-Methylbutanal	921	44,58,41,43	ST, MS
Alkanes			
Pentane	500	41, 42, 57,72	ST, MS
Hexane	600	57, 41, 56,42	ST, MS
Heptane	700	43,57,71,41	ST, MS
Octane	800	43,57,85,71	ST, MS
Undecane	1100	71,43,57,85	ST, MS
Dodecane	1200	57,43,71,85	ST, MS
Esters			
Ethyl acetate	894	43,45,61,70	MS
Ethyl butanoate	1052	43,60,71,88,100	MS
Ethyl 2-methylbutanoate	1070	57,74,85,102,115	ST, MS
Ethyl 3-methylbutanoate	1084	60,70,88,115,130	ST, MS
Ethyl hexanoate	1246	55,60,70,88,99,115	MS
Ketones			
2-Propanone	819	58,43,42,39	MS
2-Butanone	907	43,72,57	ST, MS
2-Pentanone	985	43,41,86,57	ST, MS
2-Heptanone	1198	43,58,71,85	ST, MS
2-Octanone	1300	43,58,71,59	ST, MS
2,3-Pentanedione	1081	100,42,43	ST, MS
3-Hydroxy-2-butanone	1303	43,45,73,88	MS
4-Methyl-2-pentanone	1018	43,57,100	MS
5-Methyl-3-heptanone	1170	57,72,85,114	MS
3-Ethylcyclopentanone	1348	70,83,97,112	MS
Benzene compounds			
Methylbenzene	1055	91,92,65,93	MS
Ethylbenzene	1131	91,106,65,51	MS
3-Ethyl-phenol	1232	106,122	MS
Styrene	1272	104,103,78,77	ST, MS
Ethyl styrene 1	1468	91,117,132,115	MS
Ethyl styrene 2	1476	91,117,132,115	MS
Benzaldehyde	1556	77,105,106,51	ST, MS

(continued on next page)

Table 1 (*continued*)

Compound	LRI[a]	QI[b]	ID[c]
Benzonitrile	1644	50,76,103	MS
4-Ethyl-benzaldehyde	1791	91,105,119,134	MS
Benzenemethanol	1921	51,79,91,108	MS
Phenol	2037	66,94	MS
4-Methyl-phenol	2093	77,90,107	ST, MS
o-Xylene	1199	91,106,105,77	ST, MS
m-Xylene	1157	91,106,105,77	ST, MS
p-Xylene	1150	91,106	ST, MS
3-Phenyl-2-propenal	1887	51,77,103	MS
4-Phenyl-3-buten-2-one	2026	77,103,131,146	MS
2-Phenoxy-ethanol	2175	77,90,107	MS
3-Phenoxy-1-propanol	2079	77,94,108,152	MS
Naphthalene	1800	51,64,128	MS
Sulfur compounds, non-cyclic			
Methanethiol	678	47,48,45,46	MS
Carbon disulfide	729	76,78,77,64	MS
Sulfur dioxide	849	48,64,66	MS
Dimethyl sulfide	747	35,47,61,62	MS
Dimethyl disulfide	1086	94,79,46	ST, MS
Dimethyl trisulfide	1402	126,45,47,79	ST, MS
Dimethyl sulfoxide	1624	45,63,78	MS
Furanes			
2-Methylfuran	871	51,81,82	ST, MS
3-Methylfuran	899	53,81,82	MS
2,5-Dimethylfuran	954	53,67,81,95	MS
2,3,5-Trimethylfuran	1072	52,67,81,93	MS
2,3-Dihydro-4-methylfuran	1216	50,55,69,84	MS
2-Pentylfuran	1240	81,82,95,53,138	ST, MS
Furanones			
2(3H)-Dihydrofuranone	1675	42,56,86	ST, MS
2(3H)-5-Ethyldihydrofuranone	1756	56,70,85	ST, MS
Pyrazines			
Pyrazine	1229	53,80	MS
Methylpyrazine	1285	40,53,67,94	ST, MS
2,6-Dimethylpyrazine	1346	53,67,81,93	MS
Terpenes			
α-Pinene	1032	79,93,105,121,136	MS
DL-Limonene	1209	68,79,93,107,121,136	ST, MS
Miscellaneous			
Ethyl ether	620	45,59,74	MS
2-Methylthiazole	1255	58,99	MS
p-Nitrophenyl hexanoate	1696	43,55,71,99	MS
Pyridine	1204	52,64,79	MS

[a] LRI: Linear retention indexes, calculated in relation to the retention time of n-alkane (C5–C20) series.
[b] QI: Ions used for quantification.

Table 2
Levels[1] of the 13 individual volatile compounds significantly influenced by water activity (a_w)[2] in untreated and HPP-treated Serrano hams.

Compounds	Untreated ham			HPP-treated ham			*P*[3]
	Low a_w	Medium a_w	High a_w	Low a_w	Medium a_w	High a_w	a_w
Propanoic acid	18.49	13.38	12.30	17.25	12.54	10.98	*
Hexanoic acid	323.22	206.68	201.57	301.40	189.78	182.10	**
Ethanol	429.31	730.36	804.38	401.67	755.23	781.40	***
2-Propanol	350.28	660.50	721.98	382.96	698.39	742.16	*
1-Methoxy-2-propanol	49.89	95.66	93.42	51.57	93.18	84.78	**
Ethyl acetate	51.36	65.54	76.35	41.49	59.86	58.83	*
3-Ethyl cyclopentanone	18.31	9.83	11.16	18.84	11.14	10.34	*
3-Ethyl phenol	28.69	11.25	9.63	31.33	11.38	8.28	**
3-Phenoxy-1-propanol	36.98	16.87	13.70	40.12	16.05	11.97	*
Dimethyl sulfide	11.93	19.14	24.86	12.32	14.31	18.31	*
2-Methyl furan	31.48	14.49	14.89	71.80	42.89	31.33	**
2,5-Dimethyl furan	75.86	31.79	37.81	71.80	42.89	31.33	*
5-Ethyl dihydro-2-(3H)furanone	66.18	45.71	46.58	66.62	48.86	43.54	*

[1] Levels are expressed as sums of the abundances of characteristic ions, multiplied by 10^{-5}.
[2] Low a_w was <0.853, medium a_w was within the range 0.853–0.864, and high a_w was >0.864.
[3] Statistical significance in the analysis of variance was: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Table 3
Levels[1] of the 23 individual volatile compounds significantly influenced by salt content[2] in untreated and HPP-treated Serrano hams.

Compounds	Untreated ham			HPP-treated ham			P^3
	Low salt	Medium salt	High salt	Low salt	Medium salt	High salt	Salt
1-Butanol	56.49	71.41	139.89	56.54	70.10	146.18	***
1-Pentanol	263.87	389.47	851.25	276.10	373.32	810.60	***
1-Octanol	2.43	3.34	7.53	2.82	3.22	6.88	***
2-Butanol	41.83	56.70	88.27	41.26	48.85	92.44	*
2-Pentanol	194.75	370.00	781.92	196.10	264.08	756.06	*
1-Penten-3-ol	116.78	130.36	218.57	115.23	123.95	218.18	**
2-Methyl-2-buten-1-ol	8.95	8.82	18.05	9.22	8.35	18.60	**
2-Butoxyethanol	528.62	751.54	1501.14	581.99	716.70	1543.21	***
2-Methylbutanal	565.55	397.13	225.57	467.36	369.12	176.50	**
3-Hydroxy-2-butanone	218.80	314.96	522.69	235.07	313.44	591.52	*
4-Methyl-2-pentanone	2.95	3.98	7.17	3.37	3.86	7.26	***
Methylbenzene	107.45	109.49	160.76	111.54	106.31	154.07	*
Benzenemethanol	141.45	131.57	94.08	170.85	128.12	111.54	*
4-Methylphenol	5.69	7.56	15.36	5.83	6.82	14.51	***
3-Phenoxy-1-propanol	35.09	21.52	5.96	33.89	23.02	5.59	*
Methanethiol	36.64	37.49	22.78	54.48	55.74	40.37	*
Dimethyl trisulfide	48.98	38.95	14.83	26.74	29.63	7.61	*
2-Methylfuran	27.22	21.44	8.29	28.95	18.89	8.64	*
Methylpyrazine	33.30	30.17	14.92	36.51	28.45	14.99	***
2,6-Dimethylpyrazine	124.90	100.51	23.51	136.50	98.44	23.27	***
2-Methylthiazole	3.91	3.48	1.73	4.19	3.39	1.90	***
p-Nitrophenyl hexanoate	30.11	36.66	73.48	29.81	36.95	61.16	*
Pyridine	24.22	26.86	46.21	25.93	24.92	48.03	***

[1] Levels are expressed as sums of the abundances of characteristic ions, multiplied by 10^{-5}.
[2] Low salt content was <4.83%, medium salt content was within the range 4.83–6.13%, and high salt content was >6.13%.
[3] Statistical significance in the analysis of variance: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

the packaging material by some authors (Rivas-Cañedo, Fernández-García, & Nuñez, 2009b; Stahnke, 1994).

The ripening process of ham involves complex biochemical reactions with the participation of dozens of muscle enzymes, mainly of proteolytic and lipolytic nature, that generate non-volatile and volatile compounds which finally contribute to the development of flavor. Most of the volatile compounds are the result of chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids, while others result from the Strecker degradation of free amino acids and Maillard reactions (Toldrá, 1998). Sodium chloride, nitrate and phosphate have been shown to influence the activity of muscle proteases (Sárraga, Gil, Arnau, Monfort, & Cussó, 1989) while curing agents and a_w affected the lipolytic activity in muscle and adipose subcutaneous tissue (Motilva & Toldrá, 1993). Also, the genetic and chemical characteristics of the raw material may influence the formation of volatile compounds and the flavor characteristics of ham (Berdagué, Bonnaud, Rousset, & Touraille, 1993; Ramírez & Cava, 2007). The contribution of microorganisms to the formation of volatile compounds in ham is difficult to ascertain, as the use of a "sterile ham" in experiments would be necessary to exclude their activity (Hinrichsen & Pedersen, 1995). According to these authors, microorganisms contributing to Parma ham flavor through the catabolism of amino acids should possess a secondary metabolism capable of generating methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters and dimethyl trisulfide in a distinct balance. Differences in the chemical composition of ham are expected to affect both microbial growth and metabolism.

In the present work, the effect of chemical composition on the volatile fraction of Serrano ham was more marked on acids, alcohols,

Table 4
Levels[1] of the 18 individual volatile compounds significantly influenced by salt-in-lean (S/L) ratio[2] in untreated and HPP-treated Serrano hams.

Compounds	Untreated ham			HPP-treated ham			P^3
	Low S/L	Medium S/L	High S/L	Low S/L	Medium S/L	High S/L	S/L
1-Butanol	60.03	68.69	139.89	59.15	68.09	146.18	***
1-Pentanol	277.08	379.31	851.25	277.19	372.47	810.60	***
1-Octanol	2.38	3.38	7.53	2.98	3.09	6.88	***
1-Penten-3-ol	121.68	126.59	218.57	117.99	121.83	218.18	***
2-Methyl-2-buten-1-ol	9.54	8.36	18.05	9.63	8.04	18.60	**
2-Butoxyethanol	574.80	716.02	1501.14	609.93	695.21	1543.21	***
2-Methylbutanal	556.72	403.92	225.57	459.48	375.18	176.50	**
3-Hydroxy-2-butanone	221.96	312.52	522.69	233.97	314.28	591.52	*
4-Methyl-2-pentanone	2.97	3.97	7.17	3.44	3.81	7.26	***
Benzenemethanol	106.78	110.00	160.76	106.58	110.12	154.07	*
4-Methylphenol	5.85	7.44	15.36	5.74	6.90	14.51	***
Methanethiol	36.09	37.91	22.78	54.96	55.36	40.37	*
Dimethyl trisulfide	51.12	37.30	14.83	25.56	30.54	7.61	*
Methylpyrazine	33.86	29.75	14.92	37.01	28.06	14.99	***
2,6-Dimethylpyrazine	126.87	98.99	23.51	136.31	98.58	23.27	***
2-Methylthiazole	4.00	3.41	1.73	4.15	3.42	1.90	***
p-Nitrophenyl hexanoate	31.36	35.70	73.48	31.87	35.37	61.16	**
Pyridine	24.39	26.73	46.21	26.34	24.61	48.03	***

[1] Levels are expressed as sums of the abundances of characteristic ions, multiplied by 10^{-5}.
[2] Low salt-in-lean ratio was <0.050, medium salt-in-lean ratio was within the range 0.050–0.066, and high salt-in-lean ratio was >0.066.
[3] Statistical significance in the analysis of variance was: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Table 5
Levels[1] of the 28 individual volatile compounds significantly influenced by intramuscular fat content[2] in untreated and HPP-treated Serrano hams.

Compounds	Untreated ham			HPP-treated ham			P^3
	Low fat	Medium fat	High fat	Low fat	Medium fat	High fat	Fat
Propanoic acid	11.87	14.51	18.80	11.68	11.07	19.50	**
Hexanoic acid	208.64	219.42	325.12	184.81	184.27	333.16	**
Octanoic acid	27.30	34.16	43.43	22.76	30.44	42.86	*
1-Butanol	112.29	77.39	48.25	112.86	75.10	54.06	**
1-Pentanol	683.24	389.65	232.33	612.92	388.69	283.81	*
2-Propanol	689.19	718.59	237.94	876.46	602.17	251.75	***
2-Butanol	77.24	60.01	32.95	77.69	50.50	35.58	*
2-Pentanol	727.81	310.43	101.30	624.16	270.21	106.04	**
2-Methyl-2-buten-1-ol	16.74	9.62	5.06	16.52	9.57	5.50	***
2-Butoxyethanol	1254.99	849.28	302.15	1313.13	812.56	319.61	***
1-Methoxy-2-propanol	94.80	97.88	46.05	102.10	73.81	44.00	**
2-(2-Ethoxyethoxy)-ethanol	73.39	72.90	56.81	77.00	63.57	59.14	*
2-Methylbutanal	311.25	414.08	552.32	219.08	313.68	605.91	**
2,3-Pentanedione	3.39	4.19	5.51	2.95	4.52	5.99	*
4-Methyl-2-pentanone	5.75	4.00	3.03	6.10	3.66	3.42	**
Phenol	16.25	18.35	24.22	17.26	16.46	26.76	**
3-Ethylphenol	4.87	14.17	35.59	5.38	12.20	39.17	***
4-Methylphenol	12.80	7.89	4.39	12.12	7.14	4.59	***
2-Phenoxyethanol	10.45	11.01	9.02	10.73	9.16	9.36	*
3-Phenoxy-1-propanol	7.04	20.05	46.81	7.49	16.49	51.68	**
Benzonitrile	8.94	8.00	5.42	8.99	6.70	6.07	**
Naphthalene	7.37	7.01	4.93	7.51	6.04	4.68	*
Dimethyl trisulfide	19.28	37.49	59.44	13.64	23.58	37.04	*
2-Methylfuran	10.97	19.15	34.69	10.39	15.47	38.90	***
2,5-Dimethylfuran	34.09	44.25	74.86	30.90	36.87	87.89	**
2,6-Dimethylpyrazine	53.20	95.20	135.98	51.63	92.36	152.96	***
2-Methylthiazole	2.44	3.41	4.01	2.47	3.46	4.26	**
Pyridine	39.83	30.45	17.72	40.35	30.42	17.63	**

[1] Levels are expressed as sums of the abundances of characteristic ions, multiplied by 10^{-5}.
[2] Low fat content was <3.56%, medium fat content was within the range 3.56–6.99%, and high fat content was >6.99%.
[3] Statistical significance in the analysis of variance was: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

branched-chain aldehydes, ketones, benzene compounds, sulfur compounds and miscellaneous compounds than on the rest of the chemical groups. Salt content, salt in lean ratio and intramuscular fat content had a significant influence on a higher number of volatile compounds than a_w.

Regarding carboxylic acids, formation of propanoic and hexanoic was enhanced in low a_w hams and that of propanoic, hexanoic and octanoic in high fat content hams. Linear aliphatic acids may derive from the hydrolysis of triglycerides and phospholipids and from lipid oxidation reactions (Ruiz, García, Muriel, Andrés, & Ventanas, 2002), which were probably favored under the abovementioned conditions. Short chain acids (<6 carbon atoms) are important aroma compounds in meat products due to their low threshold values and characteristic odor notes (Stahnke, 1994).

With respect to alcohols, formation of ethanol, 2-propanol and 1-methoxy-2-propanol was enhanced in high a_w hams, formation of 1-butanol, 1-pentanol, 1-octanol, 2-butanol, 2-pentanol, 1-penten-3-ol, 2-methyl-2-buten-1-ol and 2-butoxyethanol in high NaCl content hams, formation of 1-butanol, 1-pentanol, 1-octanol, 1-penten-3-ol, 2-methyl-2-buten-1-ol and 2-butoxyethanol in high salt in lean ratio hams, and that of 1-butanol, 1-pentanol, 2-propanol, 2-butanol, 2-pentanol, 2-methyl-2-buten-1-ol, 2-butoxyethanol, 2-(2-ethoxyethoxy)-ethanol and 1-methoxy-2-propanol in low fat content hams. Linear alcohols and some secondary alcohols such as 1-penten-3-ol come from the oxidation of polyunsaturated fatty acids while ethanol, 2-butanol and branched-chain alcohols mostly derive from microbial metabolism (Flores et al., 1997; Stahnke, 1995).

Table 6
Levels[1] of the 8 individual volatile compounds significantly influenced by high pressure processing (HPP) of Serrano ham.[2]

Compounds	Untreated ham	HPP-treated ham
Ethyl acetate	64.78[b]	53.36[a]
Ethyl butanoate	14.10[b]	9.83[a]
Ethyl 2-methylbutanoate	3.85[b]	2.93[a]
Ethyl 3-methylbutanoate	3.22[b]	2.44[a]
Methanethiol	33.77[a]	51.73[b]
Sulfur dioxide	6.98[a]	14.93[b]
Dimethyl disulfide	239.62[b]	84.77[a]
Dimethyl trisulfide	36.67[b]	23.53[a]

[a,b] Means on the same row bearing different superscripts differ ($P < 0.05$).
[1] Levels are expressed as the sums of the abundances of characteristic ions, multiplied by 10^{-5}.
[2] Ham samples (30 untreated samples and 30 HPP-treated samples) were analyzed in triplicate.

The only aldehyde affected by chemical composition was 2-methylbutanal which was found at higher levels in hams of low NaCl content, low salt in lean ratio and high fat content. Aldehydes derive from fatty acids and amino acids (Ordoñez, Hierro, Bruna, & De la Hoz, 1999). The major route for 2-methylbutanal seems to be the oxidative deamination–decarboxylation of leucine via the Strecker-degradation carried out by some microorganisms (García et al., 1991; Hinrichsen & Pedersen, 1995).

Ethyl acetate, the only ester affected by ham chemical composition, increased at high a_w value. Short chain fatty acid esters such as acetates, propanoates and butanoates show fruity notes and have a low odor threshold (Stahnke, 1995).

Regarding ketones, formation of 3-ethylcyclopentanone was enhanced in low a_w hams, formation of 3-hydroxy-2-butanone and 4-methyl-2-pentanone in high NaCl content hams, formation of 3-hydroxy-2-butanone and 4-methyl-2-pentanone in high salt in lean ratio hams, that of 2,3-pentanedione in high fat content hams and that of 4-methyl-2-pentanone in low fat content hams. Ketones are generally derived from lipid oxidation and β-oxidation of free fatty acids by molds, although hydroxy-2-butanone is mostly produced from carbohydrates by lactic acid bacteria (Berdagué et al., 1993; Kinsella & Hwang, 1976).

The benzene compounds 3-ethyl-phenol and 3-phenoxy-1-propanol increased in low a_w hams, while methylbenzene and 4-methyl-phenol

increased in high NaCl content hams and benzenemethanol and 3-phenoxy-1-propanol decreased. Benzenemethanol and 4-methylphenol increased in high salt in lean ratio hams, while phenol, 3-ethylphenol and 3-phenoxy-1-propanol increased in high fat content hams and benzonitrile, 2-phenoxy-ethanol and 4-methyl-phenol decreased. Alkylbenzenes derive from the cyclization of long chain unsaturated hydrocarbons, which are oxidative decomposition products of fatty acids (Min, Ina, Peterson, & Chang, 1977).

Regarding sulfur compounds, formation of dimethyl sulfide was enhanced in high a_w hams, formation of methanethiol and dimethyl trisulfide in low NaCl content hams, formation of methanethiol and dimethyl trisulfide in low salt in lean ratio hams, and that of dimethyl trisulfide in high fat content hams. Dimethyl trisulfide mostly comes from the catabolism of sulfur-containing amino acids (Hinrichsen & Pedersen, 1995; Sabio et al., 1998). Bacteria as well as molds are capable of metabolizing sulfur-containing amino acids (Berger, Khan, Molimard, Martin, & Spinnler, 1999; Dias & Weimer, 1998), and the wild fungal population of dry-cured ham has been shown to correlate with some sulfur volatile compounds, particularly at the outer muscle, although these compounds can also be generated without microbial contribution (Martín et al., 2006). Sulfur-containing volatiles are among the main compounds responsible for meaty notes in dry-cured ham (Carrapiso et al., 2002).

The miscellaneous compounds 2-methylfuran, 2,5-dimethylfuran and 5-ethyldihydro-2(3H) furanone increased in low a_w hams, while pyridine and *p*-nitrophenyl hexanoate increased in high NaCl content hams and 2-methylfuran, 2-methylthiazole, methylpyrazine and 2,6-dimethylpyrazine decreased. Pyridine and *p*-nitrophenyl hexanoate increased in high salt in lean ratio hams and 2-methylthiazole, methylpyrazine and 2,6-dimethylpyrazine decreased. 2-Methylfuran, 2,5-dimethylfuran, 2-methylthiazole and 2,6-dimethylpyrazine increased in high fat content hams while naphthalene and pyridine decreased. Volatile compounds such as furanes, furanones and pyrazines may be generated in dry-cured ham by the Maillard reactions taking place in foods containing protein and carbohydrate reducers or carbonyl compounds (Ordoñez et al., 1999), reactions which are favored by the conditions present during ham ripening. In addition, selected strains of *Penicillium chrysogenum* have been shown to contribute to the formation of pyrazines in experimental dry-cured ham (Martín et al., 2006).

High a_w values, which generally enhance microbial growth and metabolism, show a variable effect on the activity of enzymes. Enzymes in pork muscle and adipose subcutaneous tissue have different responses to curing agents and changes in a_w (Motilva, Toldrá, & Flores, 1992). In muscle, the activity of neutral and basic lipases strongly decreased at low a_w values while acid lipase was strongly activated, acid esterase was slightly activated, and neutral esterase was slightly inhibited. In adipose tissue, of low a_w value, neutral lipase was slightly activated in the complete a_w range (0.98–0.62) investigated while basic lipase was strongly activated at a_w values around 0.80 and esterases were inhibited as a_w decreased. As above mentioned, the effect of a_w value on ham microorganisms is difficult to ascertain (Hinrichsen & Pedersen, 1995). In the present work, high a_w hams exhibited higher levels of volatile compounds of microbial origin such as ethanol, 2-propanol, 1-methoxy-2-propanol, ethyl acetate and dimethyl sulfide. On the other hand, the formation of some volatile compounds coming from enzymatic activity was enhanced in low a_w hams (Table 2).

A high salt content influences lipid oxidation in foods as well as reactions of microbial origin, most probably by regulating the activity of different enzymes. Dry-cured hams of low NaCl content showed a higher proportion of polyunsaturated fatty acids from phospholipids than those of high NaCl content (Coutron-Gambotti et al., 1999). Hexanal reached lower levels in the *Semimembranosus* muscle, but higher levels in the *Biceps femoris* muscle, of 6% NaCl Iberian ham than in the respective muscles of 3% NaCl ham (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004a). Nevertheless, the same authors (Ventanas et al., 2008) concluded that salt content had no effect on the volatile fraction of Iberian ham, with the only exception of 2-pentylfuran which showed lower levels in 6% NaCl hams at day 177. Higher levels of branched-chain aldehydes were found in bacon with higher salt content, manufactured by a traditional procedure (Andersen & Hinrichsen, 1995). In the present work, there was no significant effect of NaCl content on linear aldehydes, originating from lipid oxidation reactions. However, the level of 2-methylbutanal was higher in hams of low NaCl content, possibly favored by an increase in the concentration of free amino acids as a consequence of enhanced proteolysis (Table 3).

The contribution of fat and lipolysis to the flavor of dry-cured ham is crucial. Free fatty acids generated by the action of lipases and phospholipases are oxidized to aldehydes, alkanes, alcohols, ketones, esters and other volatile compounds by chemical (auto-oxidation) or enzymatic (β-oxidation) reactions (Toldrá & Flores, 1998). Also, intramuscular fat contributes to the juiciness of meat products by coating the tongue, teeth and other parts of the mouth and stimulating saliva secretion (Ruiz et al., 2002). In the particular case of dry-cured ham, which suffers a notable dehydration during ripening, intramuscular fat itself plays an important role in maintaining the juiciness of the product (Ruiz, Ventanas, Cava, Timón, & García, 1998). To our knowledge, there is no available information on the effect of ham fat content on the formation of volatile compounds. In the present work, fat content affected not only the levels of volatile compounds coming from lipid oxidation but also those of some compounds generated through amino acid degradation. Impact aroma compounds such as 2-methylbutanal, 2,3-pentanedione and dimethyl trisulfide were found at higher levels in hams of high fat content (Table 5).

HPP treatment of dry-cured ham influences radical formation and lipid oxidation (Andrés, Møller, Adamsen, & Skibsted, 2004c), what might bring about changes in volatile formation. In the present study, HPP of Serrano ham affected significantly the levels of only 4 esters and 4 sulfur compounds (Table 6). Esters are enzymatically formed by esterification of alcohols (products of lipid oxidation) and carboxylic acids while sulfur compounds derive from sulfur-containing amino acids such as cysteine, cystine and methionine via the Strecker degradation to thiols, which are oxidized to disulfides, or can be generated by microorganisms (Martín et al., 2006). HPP treatment may affect the levels of volatile compounds in dry-cured meat products. Oxidation reactions were promoted in Iberian ham by HPP, the effect being proportionally increased at higher pressures (Andrés et al., 2004c). Treatment of Iberian ham at 600 MPa enhanced significantly the formation of linear aldehydes (Fuentes et al., 2010). In dry-cured pork loin subjected to 300, 350 or 400 MPa for 10 min at 20 °C and then stored at 4 °C in the dark for up to 45 days, linear aldehydes were not affected by HPP and only 2-pentanone increased significantly after treatment (Campus, Flores, Martinez, & Toldrá, 2008). In sliced Serrano ham treated at 400 MPa for 10 min at 12 °C, ethyl pentanoate was the only ester affected by HPP, with no significant influence on sulfur compounds and a marked decrease in the levels of alkanes (Rivas-Cañedo et al., 2009a). The higher pressure level (600 MPa) applied in the present study may be responsible for the different effects on the volatile fraction of Serrano ham. HPP of meat products not only accelerates the formation and breakdown of hydroperoxides and the reactions involved in lipid oxidation but it can also modify the structure of enzymes and their substrates (Cheftel & Culioli, 1997). Those changes may enhance or diminish the activity of enzymes, which could justify the variable results obtained by different authors and in the present study. The reduction of the levels of esters, which impart fruity odor notes, and of two sulfur compounds, with rotten egg, sewage-like and burnt notes, observed in the present work might modify the aroma of HPP-treated Serrano dry-cured ham.

5. Conclusions

Chemical composition of Serrano ham influenced the levels of a considerable number of volatile compounds. High a_w hams showed higher levels of 5 volatile compounds probably originating from microbial

metabolism than low a_w hams while low a_w hams had higher levels of 8 volatile compounds derived from lipid oxidation reactions. High NaCl content enhanced the formation of 14 volatile compounds, mostly coming from lipid oxidation, probably by acting as a regulator of enzymatic activity, while 9 volatile compounds derived from the Strecker reactions and from microbial metabolism were found at higher levels in low NaCl hams. High fat content of Serrano ham was associated with higher levels of 13 volatile compounds coming from lipid oxidation such as carboxylic acids while 14 volatile compounds, mostly alcohols, were found at higher levels in low fat content hams. HPP treatment of Serrano ham exerted a moderate effect on its volatile fraction, probably because of the low a_w values and the high chemical stability of the product. Esters and sulfur compounds were the chemical groups most affected by HPP treatment.

Acknowledgments

The authors wish to thank E. Fulladosa, P. Gou and J. Arnau (IRTA, Monells, Spain) for the selection and processing of hams, the Spanish Ministry of Science and Innovation for project RTA2010-00029-C04-04 which supported the experimental work, and INIA for funding the Ph. D. grant of N. Martínez-Onandi.

References

Andersen, H. J., & Hinrichsen, L. L. (1995). Changes in curing agents, microbial counts and volatile compounds during processing of green bacon using two different production technologies. *Journal of the Science of Food and Agriculture*, *68*, 477–487.

Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2004a). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, *84*, 375–381.

Andres, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2007). Effect of salt content and processing conditions on volatile compounds formation throughout the ripening of Iberian ham. *European Food Research and Technology*, *225*, 677–684.

Andrés, A. I., Cava, R., Ventanas, S., Thovar, V., & Ruiz, J. (2004b). Sensory characteristics of Iberian ham: Influence of salt content and processing conditions. *Meat Science*, *68*, 45–51.

Andrés, A. I., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2004c). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology*, *219*, 205–210.

AOAC (2000). *Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., et al. (1992). Flavor compounds of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *40*, 2389–2394.

Berdagué, J. L., Bonnaud, N., Rousset, S., & Touraille, C. (1993). Influence of pig crossbreed on the composition, volatile compound content and flavor of dry cured ham. *Meat Science*, *34*, 119–129.

Berdagué, J. L., Denoyer, C., Le Quéré, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *39*, 1257–1261.

Berger, C., Khan, J. A., Molimard, P., Martin, N., & Spinnler, H. E. (1999). Production of sulfur flavors by ten strains of *Geotrichum candidum*. *Applied and Environmental Microbiology*, *65*, 5510–5514.

Campus, M., Flores, M., Martinez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, *80*, 1174–1181.

Carrapiso, A. I., Ventanas, J., & García, C. (2002). Characterization of the most odor-active compounds of Iberian ham headspace. *Journal of Agricultural and Food Chemistry*, *50*, 1996–2000.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, *46*, 211–236.

Coutron-Gambotti, C., Gandemer, G., Rousset, S., Maestrini, O., & Casabianca, F. (1999). Reducing salt content of dry-cured ham: Effect on lipid composition and sensory attributes. *Food Chemistry*, *64*, 13–19.

Dias, B., & Weimer, B. (1998). Conversion of methionine to thiols by lactococci, lactobacilli, and brevibacteria. *Applied and Environmental Microbiology*, *64*, 3320–3326.

Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry*, *45*, 2178–2186.

Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method from the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, *85*, 506–514.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry*, *41*, 23–32.

Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, *5*, 451–457.

Hinrichsen, L. L., & Pedersen, S. B. (1995). Relationship among flavor, volatile compounds, chemical-changes, and microflora in Italian-type dry-cured ham during processing. *Journal of Agricultural and Food Chemistry*, *43*, 2932–2940.

Kinsella, J. E., & Hwang, D. (1976). Biosynthesis of flavors by *Penicillium roqueforti*. *Biotechnology and Bioengineering*, *18*, 927–938.

Lynch, J., Liu, Y. H., Mela, D. J., & MacFie, H. J. H. (1993). A time intensity study of the effect of oil mouthcoatings on taste perception. *Chemical Senses*, *182*, 121–129.

Lorido, L., Estévez, M., Ventanas, J., & Ventanas, S. (2015). Salt and intramuscular fat modulate dynamic perception of flavour and texture in dry-cured hams. *Meat Science*, *107*, 39–48.

Luna, G., Aparicio, R., & García-González, D. R. (2006). A tentative characterization of white dry-cured hams from Teruel (Spain) by SPME–GC. *Food Chemistry*, *97*, 621–630.

Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology*, *110*, 8–18.

Min, D. B. S., Ina, K., Peterson, R. J., & Chang, S. S. (1977). The alkylbenzenes in roast beef. *Journal of Food Science*, *42*, 503–505.

Motilva, M. J., & Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung*, *196*, 228–232.

Motilva, M. J., Toldrá, F., & Flores, J. (1992). Assay of lipase and esterase activities in fresh pork meat and dry-cured ham. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *195*, 446–450.

Ordoñez, J. A., Hierro, E. M., Bruna, J. M., & De la Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition*, *39*, 329–367.

Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry*, *55*, 1923–1931.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009a). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing. Effect of the packaging material. *Meat Science*, *82*, 162–169.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009b). Volatile compounds in fresh meats subjected to high pressure processing: Effect of the packaging material. *Meat Science*, *81*, 321–328.

Rivas-Cañedo, A., Juez-Ojeda, C., Nuñez, M., & Fernández-García, E. (2011). Effects of high-pressure processing on the volatile compounds of sliced cooked pork shoulder during refrigerated storage. *Food Chemistry*, *124*, 749–758.

Rohlf, F. J., & Sokal, R. R. (1969). *Statistical tables (pp. 157–158)*. San Francisco: W. H. Freeman and Company.

Ruiz, J., García, C., Muriel, E., Andrés, A. I., & Ventanas, J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science*, *61*, 347–354.

Ruiz, J., Ventanas, J., Cava, R., Timón, M. L., & García, C. (1998). Sensory characteristics of Iberian ham: Influence of processing time and slice location. *Food Research International*, *31*, 53–58.

Sabio, E., Vidal-Aragón, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from South European countries. *Food Chemistry*, *61*, 493–503.

Sárraga, C., Gil, M., Arnau, J., Monfort, J. M., & Cussó, R. (1989). Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Science*, *25*, 241–249.

Shahidi, F., Rubin, L. J., & Dsouza, L. A. (1986). Meat flavor volatiles — A review of the composition, techniques of analysis, and sensory evaluation. *Critical Reviews in Food Science and Nutrition*, *24*, 141–243.

Stahnke, L. H. (1994). Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Science*, *38*, 39–53.

Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels — Part II. Volatile components. *Meat Science*, *41*, 193–209.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science*, *49*, S101–S110.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, *38*, 331–352.

Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Journal of Food Science*, *57*, 813–815.

Ventanas, S., Estevez, M., Andrés, A. I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME–DED. *Meat Science*, *79*, 172–180.

3. Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage

Artículo publicado en *Meat Science* 122 (2016) 101-108

Meat Science 122 (2016) 101–108

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage

N. Martínez-Onandi, A. Rivas-Cañedo, A. Picon, M. Nuñez *

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, Madrid, 28040, Spain

ARTICLE INFO

Article history:
Received 5 March 2016
Received in revised form 29 June 2016
Accepted 31 July 2016
Available online 04 August 2016

Keywords:
Serrano dry-cured ham
Volatile compound
High pressure processing
Water activity
Salt
Intramuscular fat

ABSTRACT

One hundred and three volatile compounds were detected by solid-phase microextraction followed by gas chromatography–mass spectrometry in 30 ripened Serrano dry-cured hams, submitted or not to high pressure processing (HPP) and afterwards held for 5 months at 4 °C. The effect of ham physicochemical parameters and HPP (600 MPa for 6 min) on volatile compounds was assessed. Physicochemical parameters primarily affected the levels of acids, alcohols, alkanes, esters, benzene compounds, sulfur compounds and some miscellaneous compounds. Intramuscular fat content was the physicochemical parameter with the most pronounced effect on the volatile fraction of untreated Serrano ham after refrigerated storage, influencing the levels of 38 volatile compounds while a_w, salt content and salt-in-lean ratio respectively influenced the levels of 4, 4 and 5 volatile compounds. HPP treatment affected 21 volatile compounds, resulting in higher levels of alkanes and ketones and lower levels of esters and secondary alcohols, what might affect Serrano ham odor and aroma after 5 months of refrigerated storage.

1. Introduction

Serrano dry-cured ham is a traditional Spanish meat product worldwide known due to its particular sensory characteristics, the most appreciated of which are its unique and intense aroma and taste (Ruiz, García, Muriel, Andrés, & Ventanas, 2002). Serrano ham aroma consists of many volatile compounds including aldehydes, alcohols, ketones, carboxylic acids, and hydrocarbons (Toldrá & Flores, 1998). Volatile compound formation during ripening of dry-cured ham is associated with the lipolytic-oxidative degradation of intramuscular lipids, the catabolism of amino acids resulting from the enzymatic cleavage of proteins, and the subsequent combination of lipid-derived compounds with low molecular weight nitrogen-compounds (Ventanas et al., 1992).

The presence of intramuscular fat within the muscle fiber is essential for flavor formation in dry-cured ham (Timón, Ventanas, Carrapiso, Jurado, & García, 2001). The curing salts used during ham processing play as well an important role in the quality of the end product. In particular, NaCl contributes to microbial stability through a reduction of water activity (a_w), enhances protein solubilization, influences proteolysis, lipolysis and lipid oxidation, and directly contributes to flavor (Toldrá & Flores, 1998).

Consumers are becoming more aware of the relationship between diet and health. This awareness is leading to an increased interest for natural ingredients and food products that are not only tasty but also healthy (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014). The demand for ready-to-eat products with reduced fat and salt contents is continuously increasing. However, a reduction in the salt and fat levels of dry-cured ham can compromise the quality of the final product by altering the amount of volatile compounds generated during manufacture and ripening (Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016).

High-pressure processing (HPP) is a preservation technology widely used in the meat industry. HPP consists in the submission of food products to high hydrostatic pressures at low or moderate temperatures, what results in a reduction of their microbial load (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004). Changes in color parameters (Andrés, Adamsen, Møller, Ruiz, & Skibsted, 2006; Cava, Ladero, González, Carrasco, & Ramírez, 2009), lipid oxidation (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004), and volatile profile (Rivas-Cañedo, Fernández-García, & Nuñez, 2009) of HPP-treated dry-cured ham have been reported. The effect of HPP on the volatile profile of Serrano dry-cured ham with different fat and salt contents has been recently investigated (Martínez-Onandi et al., 2016). However, there is no information on how the volatile profile of HPP-treated dry-cured ham would evolve during refrigerated storage.

The aim of the present work, was to investigate the influence of ham physicochemical parameters (a_w, salt content, salt-in-lean ratio and

* Corresponding author.

http://dx.doi.org/10.1016/j.meatsci.2016.07.027
0309-1740/

intramuscular fat content) and HPP treatment on the volatile fraction of ripened Serrano ham after refrigerated storage for 5 months at 4 °C.

2. Material and methods

2.1. Selection, manufacture and ripening of Serrano hams

Serrano hams were manufactured at the Institute of Food and Agricultural Research and Technology (IRTA, Monells, Spain), following the procedures used in the previous study (Martínez-Onandi et al., 2016). Thirty green hams from animals of different genotype (21 hams from Large White x Landrace animals and 9 from animals with a minimum of 50% Duroc breed) were chosen in order to obtain a wide range of fat contents. Average weight of green hams was 11.48 kg (SD, 0.63 kg) whereas the pH in the *Semimembranosus* muscle at 24 h post-mortem ranged from 5.4 to 5.9. Salting times varying from 7 to 15 days were applied to hams in order to achieve a wide range of salt contents. After salting, hams were washed with cold water, weighed and hung in a cold room at 3 °C and 75–80% RH to rest. During ripening the temperature was progressively increased up to 20 °C. Hams were ripened for 9 to 10 months until a total weight loss of 36% was achieved.

2.2. Sampling and high pressure processing

Two slices (approximately 150 g) from the cushion (mainly composed of the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* muscles) were obtained from each of the ripened hams and individually vacuum-packaged. One of the slices was submitted to HPP-processing at 600 MPa for 6 min at 21 °C (pressure build up time, 2.5 min; pressure release time < 2 s) in a 120 L capacity Wave 6000 equipment (Hiperbaric, Burgos, Spain) at IRTA (Monells, Spain). The second slice served as untreated control. Both ham slices were kept for 5 months at 4 °C, simulating the time-temperature conditions of commercial refrigerated storage.

2.3. Physicochemical determinations

Representative homogenates of ham slices were obtained using a mechanical grinder (IKA Labortechnik, Staufen, Germany). Water activity (a_w) was measured using an AquaLab Series 3 equipment (Decagon, Devices, Inc., Pullman, WA, USA). Chloride content was determined by the Volhard method (AOAC, 2000) and intramuscular fat content by the Folch method (Folch, Lees, & Sloane-Stanley, 1957). All analyses were performed in triplicate.

2.4. Analysis of volatile compounds

Volatile compounds were extracted by solid-phase microextraction (SPME) and analyzed by gas chromatography–mass spectrometry (GC–MS) (HP 6890-MSD HP 5973, Agilent, Palo Alto, CA, USA). Fifteen grams of Serrano ham, trimmed of the subcutaneous fat, were homogenized in a mechanical grinder (IKA Labortechnik) with 15 g of anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) and 30 μL of an aqueous solution of 534 mg/L cyclohexanone (Sigma-Aldrich, Alcobendas, Spain) as internal standard as previously described (Martínez-Onandi et al., 2016). Compound identification was carried out by injection of commercial standards, by spectra comparison using the Wiley7Nist05 Library (Wiley & Sons Inc., Germany), and/or by calculation of linear retention indexes (LRI) relative to a series of alkanes (C5-C20). The sums of the abundances of selected characteristic ions were used for the semi-quantitative determination of volatile compounds. Data thus obtained were multiplied by 10^{-5} to express the levels of volatile compounds, for easier comprehension by the reader. Each ham was analyzed in triplicate.

2.5. Statistical analysis

Data were analyzed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA). Chromatographic areas were subjected to analysis of variance with one of the physicochemical parameters (a_w, salt content, salt-in-lean ratio or fat content) or HPP treatment as main effects (one-way ANOVA). Three groups of hams with low, medium and high values for each of the physicochemical parameters were set by using the mean ± 0.5 standard deviations (SD) criterion for the separation of hams (Martínez-Onandi et al., 2016). Comparison of the levels of volatile compounds between the groups of hams was performed by using the Tukey's test, with the significance assigned at $P < 0.05$.

3. Results

One hundred and three compounds were detected in the volatile fraction of Serrano dry-cured ham by means of SPME followed by GC–MS. Table 1 lists the compounds grouped by chemical families, together with their linear retention indexes, the ions used for quantification and the method used for identification. The volatile compounds were grouped into 8 acids, 21 alcohols, 7 aldehydes, 8 alkanes, 6 esters, 9 ketones, 18 benzene compounds, 7 non-cyclic sulfur compounds and 19 miscellaneous compounds, which included 7 furanes, 2 furanones, 4 pyrazines, 2 terpenes and 4 other compounds.

3.1. Effect of ham physicochemical parameters

Water activity (a_w) of Serrano hams ranged from 0.860 to 0.899, with a mean value of 0.880 (SD, 0.010). They were grouped into 8 low a_w (<0.875), 12 medium a_w (0.875–0.884) and 10 high a_w (>0.884) hams. The levels of 4 volatile compounds in untreated samples and 9 volatile compounds in HPP-treated samples were significantly influenced by a_w. The only volatile compound significantly affected in both untreated and HPP-treated samples was 2-pentanol, which showed higher levels in medium a_w hams than in low and high a_w hams (Table 2).

NaCl concentration of hams ranged from 5.29% to 11.16%, with a mean value of 7.27% NaCl on total weight (SD, 1.36%). They were grouped into 10 hams of low (<6.59%), 12 of medium (6.59–7.95%) and 8 of high (>7.95%) salt content. Salt concentration significantly influenced the levels of 4 volatile compounds in untreated samples and of 4 volatile compounds in HPP-treated samples. Methylpyrazine, which showed higher levels in low NaCl content hams than in high NaCl content hams, was the only volatile compound significantly affected in both untreated and HPP-treated samples (Table 3).

The salt-in-lean ratio of hams ranged from 0.057 to 0.114, with a mean value of 0.078 (SD, 0.014). They were grouped into 10 hams of low (<0.071), 12 of medium (0.071–0.085) and 8 of high (>0.085) salt-in-lean ratio. Salt-in-lean ratio significantly influenced 5 volatile compounds in untreated samples and 4 volatile compounds in HPP-treated samples. Methylpyrazine and 2,6-dimethylpyrazine were the only volatile compounds significantly affected in both untreated and HPP-treated samples, reaching higher levels in low salt-in-lean ratio hams than in high salt-in-lean ratio hams (Table 4).

The intramuscular fat content of hams ranged from 2.28% to 12.47%, with a mean value of 7.01% on total weight (SD, 2.40%). They were grouped into 10 hams of low (<5.81%), 12 of medium (5.81–8.21%) and 8 of high (>8.21%) intramuscular fat content. Fat content significantly influenced the levels of 38 volatile compounds in untreated samples and 33 volatile compounds in HPP-treated samples while 25 volatile compounds were significantly affected in both untreated and HPP-treated samples. High fat content hams had higher levels of acetic, hexanoic and heptanoic acids, ethyl butanoate, ethyl hexanoate, ethyl decanoate, methylbenzene, phenol, 3-ethylphenol, 3-phenoxy-1-propanol, 2-methylfuran, 2-ethylfuran, 2,5-dimethylfuran and *p*-nitrophenyl hexanoate than low fat content hams whereas low fat content

Table 1
Volatile compounds identified in untreated and HPP-treated Serrano hams after refrigerated storage for 5 months at 4 °C.

Compound	LRI[a]	QI[b]	ID[c]
Acids			
Acetic acid	1469	43,45,60	MS
Propanoic acid	1549	45,57,74	ST, MS
Butanoic acid	1641	60,73,88	ST, MS
Pentanoic acid	1682	43,60,87	ST, MS
Hexanoic acid	1855	41,60,87	ST, MS
Heptanoic acid	1968	43, 60, 73	MS
Octanoic acid	2063	43,73,101,115	ST, MS
2-Methyl propanoic acid	1578	43,73,88	ST, MS
Alcohols			
Ethanol	941	45,46,43,41	MS
1-Propanol	1059	59, 42, 60	MS
1-Butanol	1165	56,41,43,42	ST, MS
1-Pentanol	1265	42,55,41,70	ST, MS
1-Hexanol	1364	56,55,69,41	ST, MS
1-Heptanol	1479	70,56,55,69	ST, MS
1-Octanol	1582	56,55,69,70	ST, MS
2-Propanol	934	45,43,41,59	MS
2-Butanol	1046	45,59	ST, MS
2-Pentanol	1141	45,73	ST, MS
2-Hexanol	1236	45,43,69,57	ST, MS
2-Methyl-1-propanol	1112	42,43,41,74	ST, MS
3-Methyl-1-butanol	1225	55,70,42,43	ST, MS
3-Methyl-2-butanol	1140	45, 55, 73	MS
1-Penten-3-ol	1177	57,41,43,88	MS
1-Octen-3-ol	1471	57,72,55	ST, MS
2-Methyl-3-buten-2-ol	1060	53,59,65,71	MS
2-Butoxyethanol	1431	57,75,87,100,108	MS
2-(2-Ethoxyethoxy)-ethanol	1653	45,59,72,104	MS
1-Methoxy-2-propanol	1148	45,59,75,90	MS
4-Butoxy-1-butanol	1724	57,71,103,113,129	MS
Aldehydes			
Hexanal	1097	56,57,72	ST, MS
Heptanal	1199	70,44,43,55	ST, MS
Nonanal	1413	57,98,70,82	ST, MS
Dodecanal	1739	57,82,68,96	ST, MS
2-Methylpropanal	816	41,43,72	MS
2-Methylbutanal	917	57,41,58,39	ST, MS
3-Methylbutanal	921	44,58,41,43	ST, MS
Alkanes			
Pentane	500	41, 42, 57,72	ST, MS
Hexane	600	57, 41, 56,42	ST, MS
Heptane	700	43,57,71,41	ST, MS
Octane	800	43,57,85,71	ST, MS
Decane	1000	43,57,71,85,99	MS
Undecane	1100	71,43,57,85	ST, MS
Dodecane	1200	57,43,71,85	ST, MS
Branched-chain alkane	944	57,43,71,41	MS
Esters			
Ethyl acetate	894	43,45,61,70	MS
Ethyl butanoate	1052	43,60,71,88,100	MS
Ethyl 2-methylbutanoate	1070	57,74,85,102,115	ST, MS
Ethyl 3-methylbutanoate	1084	60,70,88,115,130	ST, MS
Ethyl hexanoate	1246	55,60,70,88,99,115	MS
Ethyl decanoate	1661	88, 101, 157	MS
Ketones			
2-Propanone	819	58,43,42,39	MS
2-Butanone	907	43,72,57	ST, MS
2-Pentanone	985	43,41,86,57	ST, MS
2-Heptanone	1198	43,58,71,85	ST, MS
2-Octanone	1300	43,58,71,59	ST, MS
3-Hydroxy-2-butanone	1303	43,45,73,88	MS
4-Methyl-2-pentanone	1018	43,57,100	MS
5-Methyl-3-heptanone	1170	57,72,85,114	MS
3-Ethylcyclopentanone	1348	70,83,97,112	MS
Benzene compounds			
Methylbenzene	1055	91,92,65,93	MS
Ethylbenzene	1131	91,106,65,51	MS
Styrene	1272	104,103,78,77	ST, MS
Benzaldehyde	1556	77,105,106,51	ST, MS
4-Ethyl-benzaldehyde	1791	91,105,119,134	MS
Benzonitrile	1644	50,76,103	MS
Benzenemethanol	1921	51,79,91,108	MS
Phenol	2037	66,94	MS
4-Methyl-phenol	2093	77,90,107	ST, MS
3-Ethyl-phenol	1232	106,122	MS
o-Xylene	1199	91,106,105,77	ST, MS
m-Xylene	1157	91,106,105,77	ST, MS
p-Xylene	1150	91,106	ST, MS
3-Phenyl-2-propenal	1887	51,77,103	MS
4-Phenyl-3-buten-2-one	2026	77,103,131,146	MS
2-Phenoxy-ethanol	2175	77,90,107	MS
3-Phenoxy-1-propanol	2079	77,94,108,152	MS
Naphthalene	1800	51,64,128	MS
Sulfur compounds, non-cyclic			
Methanethiol	678	47,48,45,46	MS
Carbon disulfide	729	76,78,77,64	MS
Sulfur dioxide	849	48,64,66	MS
Dimethyl sulfide	747	35,47,61,62	MS
Dimethyl disulfide	1086	94,79,46	ST, MS
Dimethyl trisulfide	1402	126,45,47,79	ST, MS
Dimethyl sulfoxide	1624	45,63,78	MS
Furanes			
2-Methylfuran	871	51,81,82	ST, MS
3-Methylfuran	899	53,81,82	MS
2-Ethylfuran	952	53,81,96,51,82	MS
2,5-Dimethylfuran	954	53,67,81,95	MS
2,3,5-Trimethylfuran	1072	52,67,81,93	MS
2,3-Dihydro-4-methylfuran	1216	50,55,69,84	MS
2-Pentylfuran	1240	81,82,95,53,138	ST, MS
Furanones			
2(3H)-Dihydrofuranone	1675	42,56,86	ST, MS
2(3H)-5-Ethyldihydrofuranone	1756	56,70,85	ST, MS
Pyrazines			
Pyrazine	1229	53,80	MS
Methylpyrazine	1285	40,53,67,94	ST, MS
2,6-Dimethylpyrazine	1346	53,67,81,93	MS
2,3,5-Trimethylpyrazine	1437	122,42,81,39	MS
Terpenes			
Alpha-pinene	1032	79,93,105,121,136	MS
DL-Limonene	1209	68,79,93,107,121,136	ST, MS
Other compounds			
Ethyl ether	620	45,59,74	MS
2-Methylthiazole	1255	58,99	MS
p-Nitrophenyl hexanoate	1696	43,55,71,99	MS
Pyridine	1204	52,64,79	MS

[a] LRI: Linear retention indexes, calculated in relation to the retention time of n-alkane (C5–C20) series.
[b] QI: Ions used for quantification.
[c] ID: Peak identification: ST, comparison of spectra and retention time with commercial standards; MS, tentatively identified by spectra comparison using the Wiley Library.

hams showed higher levels of 2-propanol, 2-butoxyethanol, 1-methoxy-2-propanol, branched-chain alkane, 4-methyl-phenol, dimethyl sulfide and pyridine than high fat content hams. Low and medium fat content hams had higher levels of 2-butanol and 2-pentanol than high fat content hams while high fat content hams showed higher levels of hexanal than low and medium fat content hams, independently of HPP treatment. Untreated hams of low and medium fat content had higher levels of dimethyl trisulfide than untreated hams of high fat content whereas HPP-treated hams of high fat content showed higher levels of this compound than HPP-treated hams of low and medium fat content (Table 5).

3.2. Effect of high pressure processing

Twenty one out of 103 volatile compounds of Serrano ham were significantly influenced by HPP treatment (Table 6). The levels of 2-

Table 2
Levels[a] of the volatile compounds significantly influenced by water activity (a_w)[b] in untreated or HPP-treated Serrano hams after refrigerated storage for 5 months at 4 °C.

Compound	Untreated hams				HPP-treated hams			
	Low a_w ($n = 8$)	Medium a_w ($n = 12$)	High a_w ($n = 10$)	P^c	Low a_w ($n = 8$)	Medium a_w ($n = 12$)	High a_w ($n = 10$)	P^c
1-Octanol	2.09	1.16	1.65	*	2.86	2.47	2.64	ns
2-Pentanol	305.49	712.84	300.51	**	202.50	434.24	183.68	**
Hexanal	428.23	204.56	231.10	ns	410.59	152.13	231.38	*
2-Methylbutanal	36.47	30.40	35.77	ns	31.57	23.27	38.88	*
Branched-chain alkane	10.75	27.42	14.93	*	15.19	24.22	16.21	ns
3-Hydroxy-2-butanone	146.67	252.57	178.62	ns	167.97	311.98	195.48	*
Methylbenzene	107.59	91.40	111.22	ns	116.06	88.95	128.53	*
p-Xylene	3.18	2.97	3.25	ns	3.02	2.36	3.41	*
Methanethiol	30.82	27.48	29.58	ns	44.16	46.79	60.54	*
2-Methylthiazole	2.48	3.02	3.49	ns	2.74	2.83	3.77	**
p-Nitrophenyl hexanoate	32.54	9.80	12.36	ns	44.93	20.87	22.13	*
Pyridine	21.35	34.22	24.37	*	20.26	32.28	27.56	ns

[a] Levels of volatile compounds are expressed as sum of the abundances of characteristic ions, multiplied by 10^{-5}. Ham samples were analyzed in triplicate.
[b] Low a_w was <0.875, medium a_w was within the range 0.875–0.884, and high a_w was >0.884.
[c] Statistical significance was *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, non-significant.

butanol, 2-pentanol, 2-hexanol, 2-heptanol, 3-methyl-2-butanol, ethyl acetate, ethyl butanoate, dimethyl sulfide and dimethyl disulfide were significantly higher in untreated samples while the levels of 1-octanol, 1-penten-3-ol, 1-octen-3-ol, hexane, dodecane, 2-propanone, 2-butanone, 2-pentanone, 5-methyl-3-heptanone, 3-phenyl-2-propenal, methanethiol and sulfur dioxide were significantly higher in HPP-treated samples.

4. Discussion

4.1. Volatile fraction of ham after prolonged refrigerated storage

The aroma of dry-cured ham includes a large number of chemical families such as aldehydes, sulfur compounds, ketones, esters and alcohols (Carrapiso, Ventanas, & García, 2002; Flores, Grimm, Toldrá, & Spanier, 1997). In the present study, 103 volatile compounds belonging to 12 chemical groups were detected using the SPME technique in untreated and HPP-treated Serrano ham samples after refrigerated storage, a number close to the 100 volatile compounds identified in a previous work using the same technique (Martínez-Onandi et al., 2016). The 103 volatile compounds are a slightly higher number than the 84 to 93 compounds identified in Serrano ham using the purge-and-trap dynamic headspace extraction technique (Flores, Gianelli, Pérez-Juan, & Toldrá, 2007; Ramírez & Cava, 2007; Rivas-Cañedo et al., 2009) although lower than the 122 volatile compounds identified in Parma dry-cured ham using the dynamic headspace extraction technique (Barbieri et al., 1992). Eight carboxylic acids were detected in the present study, a number similar to the 7 found in a previous work (Martínez-Onandi et al., 2016) whereas other authors did not detect any carboxylic acid (Rivas-Cañedo et al., 2009; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998). Twenty-one alcohols were found in the present study whereas Sabio et al. (1998) and Berdagué, Denoyer, Le Quéré, and Semon (1991) only detected 14 and 8 alcohols, respectively. Some of the unsaturated alcohols detected in the present study such as 1-penten-3-ol and 1-octen-3-ol have strong and distinctive smells (Berdagué et al., 1991). The latter alcohol was also detected by Flores et al. (1997). Four linear aldehydes and 3 branched-chain aldehydes were found in the present study, in agreement with a previous work (Martínez-Onandi et al., 2016), while Rivas-Cañedo et al. (2009) detected 9 linear aldehydes and 6 branched-chain aldehydes. One branched-chain alkane (BCA) was found in the present work whereas Martínez-Onandi et al. (2016) and Sabio et al. (1998) did not detect any BCA. Generally, BCA compounds come from the packaging plastic material (Rivas-Cañedo et al., 2009) and they are generally considered as not contributing to meat flavor (Shahidi, Rubin, & D'Souza, 1986).

Many biochemical changes (lipolysis, proteolysis, oxidation reactions, Strecker degradation and Maillard reactions) take place during the manufacture and ripening of dry-cured ham and contribute to flavor development (Narváez-Rivas, Vicario, Constante, & León-Camacho, 2007; Toldrá & Flores, 1998). Microorganisms may generate compounds such as methyl aldehydes, secondary alcohols, methyl ketones, ethyl esters and sulfur compounds from amino acids in dry-cured ham (Hinrichsen & Pedersen, 1995). Their role in the development of Serrano ham remains unclear. The genetic traits and the chemical composition of the raw material as well as the manufacturing conditions influence the generation of volatile compounds in dry-cured ham. In addition, industrial treatments such as HPP and long refrigeration periods could affect the profile of volatile compounds in dry-cured ham.

4.2. Influence of physicochemical parameters on volatile compounds

Physicochemical parameters may influence the formation of volatile compounds during ham processing, ripening and storage through their

Table 3
Levels[a] of the volatile compounds significantly influenced by salt content[b] in untreated or HPP-treated Serrano hams after refrigerated storage for 5 months at 4 °C.

Compound	Untreated hams				HPP-treated hams			
	Low salt ($n = 10$)	Medium salt ($n = 12$)	High salt ($n = 8$)	P^c	Low salt ($n = 10$)	Medium salt ($n = 12$)	High salt ($n = 8$)	P^c
1-Butanol	34.16	46.94	49.86	*	41.47	49.09	51.04	ns
1-Penten-3-ol	86.23	101.97	126.49	*	110.64	131.75	146.92	ns
2-Propanone	394.54	487.15	365.22	*	716.17	725.52	527.04	ns
Methanethiol	28.27	30.45	27.99	ns	59.88	48.72	42.09	*
Methylpyrazine	27.97	26.42	19.74	*	32.50	27.14	20.01	*
2,6-Dimetylpyrazine	110.10	97.58	66.35	ns	127.68	101.85	71.22	*
2-Methylthiazole	3.32	3.05	2.64	ns	3.76	2.94	2.59	**

[a] Levels of volatile compounds are expressed as sum of the abundances of characteristic ions, multiplied by 10^{-5}. Ham samples were analyzed in triplicate.
[b] Low salt (NaCl) content was <6.59%, medium salt content was within the range 6.59–7.95%, and high salt content was >7.95%.
[c] Statistical significance was *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, non-significant.

Table 4
Levels[a] of the volatile compounds significantly influenced by the salt-in-lean (S/L) ratio[b] in untreated or HPP-treated Serrano hams after refrigerated storage for 5 months at 4 °C.

Compound	Untreated hams				HPP-treated hams			
	Low S/L (n = 10)	Medium S/L (n = 12)	High S/L (n = 8)	P^c	Low S/L (n = 10)	Medium S/L (n = 12)	High S/L (n = 8)	P^c
1-Butanol	32.66	46.99	49.86	*	41.15	48.72	51.04	ns
1-Penten-3-ol	80.63	104.64	126.49	*	109.21	131.12	146.92	ns
2-Propanone	390.89	482.56	365.22	*	740.10	708.23	527.04	ns
Methanethiol	28.64	30.02	27.99	ns	61.77	48.28	42.09	*
Methylpyrazine	28.73	26.02	19.74	*	33.69	26.73	20.01	*
2,6-Dimethylpyrazine	113.00	96.53	66.35	*	131.56	101.15	71.22	*
2-Methylthiazole	3.52	2.94	2.64	ns	3.93	2.89	2.59	***

[a] Levels of volatile compounds are expressed as sum of the abundances of characteristic ions, multiplied by 10^{-5}. Ham samples were analyzed in triplicate.
[b] Low S/L ratio was <0.071, medium S/L ratio was within the range 0.071–0.085, and high S/L ratio was >0.085.
[c] Statistical significance was *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, non-significant.

action on enzymes and microorganisms. A decrease in ham a_w value markedly decreases the activity of cathepsins, aminopeptidases and neutral lipases, while calpains are only slightly affected and acid lipase and acid phospholipase are quite insensitive to a decrease in a_w (Toldrá, 2006; Toldrá, Rico, & Flores, 1993). Salt, besides lowering a_w, can affect numerous chemical reactions in dry-cured ham by modulating enzyme activity (Motilva & Toldrá, 1993). However, no significant effect of salt content on hexanal formation in dry fermented sausages (Stahnke, 1995) or in Iberian ham (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2007) was observed. Intramuscular fat constitutes a substrate for the formation of volatile compounds and consequently its level might influence the volatile fraction of dry-cured meat products. Volatile compounds such as hexanol, octanal, (E,E)-2,4-heptadienal or (E)-2-decenal, derived from lipid oxidative reactions, and others such as dimethylsulfide, 3-methylbutanal or phenylacetaldehyde, coming from the degradation of certain amino acids, showed higher levels in Iberian dry-cured loins of high intramuscular fat content than in loins of low intramuscular fat content (Ventanas, Estevez, Andrés, & Ruiz, 2008).

In the present work, the influence of physicochemical parameters on the volatile fraction of Serrano ham after a 5-month refrigeration period was more marked on acids, alcohols, alkanes, ketones, benzene compounds, sulfur compounds and some miscellaneous compounds than on the rest of the chemical groups. It is noteworthy that intramuscular fat content had a significant influence on a higher number of volatile compounds than other physicochemical parameters such as a_w, salt content and salt-in-lean ratio. Considering exclusively the data of untreated hams, intramuscular fat content influenced as many as 38 volatile compounds, while a_w, salt content and salt-in-lean ratio only influenced 4, 4 and 5 volatile compounds, respectively. A less marked effect of intramuscular fat content on the formation of volatile compounds in Iberian dry-cured loins with different intramuscular fat contents, affecting only the levels of 5 aldehydes, 1 alcohol and 1 sulfur compound, was recorded (Ventanas et al., 2008). In the present study, the reduced effect of NaCl content (range, 5.29–11.16%) and a_w (range, 0.860–0.899) on volatile compounds in comparison with the effect of intramuscular fat content (range, 2.28–12.47%) might be partly explained by the relatively wider range of the latter physicochemical parameter. Values reached by those physicochemical parameters in the present study are within the standard range of commercial Serrano hams.

With respect to carboxylic acids, the highest levels of acetic, hexanoic and heptanoic acids were found in hams of high intramuscular fat content. These acids have also been detected in the headspace of Iberian ham (Ruiz, Ventanas, & Cava, 2001) and Parma ham (Barbieri et al., 1992). Higher levels of hexanoic acid in Serrano hams of high fat content than in hams of low or medium fat content have been previously reported (Martínez-Onandi et al., 2016). Linear aliphatic acids are mainly generated through lipid oxidation reactions (Ramírez & Cava, 2007). The origin of acetic acid in dry-cured ham is unclear, although some authors suggested that it could derive from Maillard reactions (Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006).

Regarding alcohols, 2-pentanol was at higher levels in medium a_w hams, 2-butanol and 2-pentanol in medium fat content hams, and 2-propanol, 2-butoxyethanol and 1-methoxy-2-propanol in low fat contents hams. Higher levels of 2-propanol and 1-methoxy-2-propanol have been reported in Serrano hams of low fat content than in hams of high fat content (Martínez-Onandi et al., 2016). Linear alcohols are products of lipid oxidative decomposition (Ramírez & Cava, 2007; Sánchez-Peña, Luna, García-González, & Aparicio, 2005; Toldrá, 1998). However, 2-alcohols and branched-chain alcohols, such as 2-propanol and 1-methoxy-2-propanol, can derive from microbial metabolism (Flores et al., 1997; Stahnke, 1994). Alcohols contribute to ham aroma with green, onion-toasted and potato-wheat notes (Toldrá & Flores, 1998), although at a lesser degree than aldehydes due to their higher odor threshold.

Ethyl butanoate and ethyl decanoate were the only two esters affected by ham chemical composition, reaching the highest levels in hams of high fat content. Esters are enzymatically formed by esterification of alcohols and carboxylic acids (García et al., 1991; Ramírez & Cava, 2007; Sabio et al., 1998). They contribute to dry-cured ham aroma imparting fruity and sweet notes, in particular short-chain fatty acids esters such as ethyl butanoate (Narváez-Rivas, Gallardo, & León-Camacho, 2012).

Benzene compounds such as phenol, 3-ethylphenol and 3-phenoxy-1-propanol were at higher levels in hams of high fat content and 4-methylphenol in hams of low fat content. Similar results were obtained in a previous work (Martínez-Onandi et al., 2016). Alkylbenzenes derive from the cyclization of long-chain unsaturated hydrocarbons (Min, Ina, Peterson, & Chang, 1977).

Regarding sulfur compounds, dimethyl sulfide was at higher levels in low fat content hams and dimethyl trisulfide in medium or high fat content hams. Sulfide compounds can originate via Strecker degradation from sulfur-containing amino acids to thiols (Sabio et al., 1998; Shahidi et al., 1986). In particular, dimethyl sulfide is formed by spontaneous degradation of *S*-methylmethionine (Spinnler, Berger, Lapadatescu, & Bonnarme, 2001). Sulfur-containing volatiles are among the main compounds responsible for the meaty notes in Iberian ham and are well-known contributors to the flavor of cooked meats (Carrapiso & García, 2004).

Among pyrazines, methylpyrazine reached higher levels in low NaCl content and low salt-in-lean ratio hams while 2,6-dimethylpyrazine was at higher levels in low salt-in-lean ratio hams. Three furanes (2-methylfuran, 2-ethylfuran and 2,5-dimethylfuran) and *p*-nitrophenyl hexanoate were at higher levels in high fat content hams and pyridine in low fat content hams. Similar results for 2,6-dimethylpyrazine, 2-methylfuran and 2,5 dimethylfuran were obtained by Martínez-Onandi et al. (2016). Pyrazines are commonly found in high-temperature cooked meats and impart nutty, roasted, or toasted aromas (Barbieri et al., 1992). In dry-cured ham, which ripens at temperatures below 30 °C, pyrazines are generated through Maillard reactions (Flores et al., 1997) which can be favored by the intense dehydration occurring during ripening. In addition, Martín et al. (2006) suggested that some of these compounds may result from microbial activity.

Table 5
Levels[a] of the volatile compounds significantly influenced by intramuscular fat content[b] in untreated or HPP-treated Serrano hams after refrigerated storage for 5 months at 4 °C.

Compound	Untreated hams				HPP-treated hams			
	Low fat (n = 10)	Medium fat (n = 12)	High fat (n = 8)	P[c]	Low fat (n = 10)	Medium fat (n = 12)	High fat (n = 8)	P[c]
Acetic acid	443.96	586.80	641.66	*	372.07	570.68	691.13	***
Propanoic acid	12.55	16.60	19.18	ns	11.22	14.92	22.98	***
Hexanoic acid	190.84	246.74	373.79	*	162.34	237.98	404.75	**
Heptanoic acid	4.90	8.71	18.40	**	5.22	8.43	18.01	***
Octanoic acid	19.00	34.47	29.23	ns	19.49	26.62	29.26	*
Ethanol	825.00	529.37	843.96	ns	474.23	325.05	906.47	**
2-Propanol	435.42	382.81	168.41	***	402.88	315.42	171.98	***
2-Butanol	75.55	99.01	40.35	**	50.34	56.50	27.77	*
2-Pentanol	532.84	599.15	185.62	*	350.40	366.61	95.55	*
2-Methyl-1-propanol	34.79	22.27	14.54	*	33.20	19.94	16.72	ns
3-Methyl-1-butanol	227.31	135.33	105.41	*	232.71	109.44	109.29	ns
3-Methyl-2-butanol	15.63	8.92	7.19	**	8.63	6.37	6.51	ns
2-Butoxyethanol	774.24	428.19	233.37	***	763.87	471.04	239.92	***
1-Methoxy-2-propanol	3.00	2.49	1.89	**	3.18	2.83	1.87	***
Hexanal	164.72	197.72	521.47	**	142.92	193.82	458.63	**
Heptanal	8.24	7.11	12.66	*	5.57	7.58	10.54	ns
Nonanal	71.21	46.39	29.62	*	40.46	42.98	36.61	ns
2-Methylbutanal	26.56	34.07	42.47	*	24.56	30.24	39.02	ns
Dodecane	42.75	31.28	10.55	**	87.69	211.93	245.98	ns
Branched-chain alkane	24.45	23.93	4.09	**	26.37	23.55	3.51	***
Ethyl acetate	61.95	56.83	98.45	ns	28.79	36.03	76.33	*
Ethyl butanoate	16.67	10.67	27.37	*	6.01	4.95	22.17	**
Ethyl hexanoate	12.61	9.47	48.95	**	4.91	5.28	53.52	**
Ethyl decanoate	3.09	2.60	5.03	*	1.93	2.11	5.78	**
2-Propanone	419.94	465.18	366.43	ns	691.02	766.76	496.61	*
2-Heptanone	129.40	278.23	481.28	*	252.42	439.92	602.24	ns
Methylbenzene	86.84	93.51	134.91	**	90.70	103.67	141.26	*
Ethylbenzene	3.80	3.98	5.97	***	4.13	4.33	5.51	ns
Phenol	11.28	12.63	22.38	***	11.80	14.35	24.54	***
3-Ethylphenol	3.88	9.99	26.65	***	2.96	9.74	26.29	***
4-Methylphenol	8.32	5.43	3.29	***	7.61	5.74	3.58	***
3-Phenoxy-1-propanol	12.10	20.83	50.09	***	10.43	22.18	51.20	***
Carbon disulfide	10.65	13.52	16.67	ns	12.48	17.32	29.00	*
Dimethyl sulfide	20.09	16.85	7.35	***	12.36	12.16	7.45	*
Dimethyl disulfide	204.91	217.57	82.81	**	53.97	84.16	67.94	ns
Dimethyl trisulfide	25.25	33.20	15.72	*	10.80	21.83	32.88	*
2-Methylfuran	10.75	17.70	23.74	***	9.36	17.21	27.21	***
2-Ethylfuran	35.82	46.12	88.07	**	32.54	52.55	87.77	**
2,5-Dimethylfuran	28.66	37.00	70.96	***	25.95	41.97	70.61	**
2(3H)-Dihydrofuranone	41.23	44.28	43.71	ns	39.18	45.32	50.31	*
2(3H)-5-Ethyldihydrofuranone	44.26	44.95	43.71	ns	39.30	45.28	68.52	*
Pyrazine	4.50	4.17	2.32	*	4.40	4.14	2.55	ns
Methylpyrazine	22.44	29.28	22.37	*	23.67	31.46	24.56	ns
2,6-Dimethylpyrazine	67.39	114.58	94.22	*	75.17	122.33	106.15	ns
p-Nitrophenyl hexanoate	4.37	11.70	39.67	**	19.94	20.61	48.07	**
Pyridine	35.52	27.25	17.86	**	34.58	26.80	19.70	*

[a] Levels of volatile compounds are expressed as sum of the abundances of characteristic ions, multiplied by 10^{-5}. Ham samples were analyzed in triplicate.
[b] Low intramuscular fat content was <5.81%, medium intramuscular fat content was within the range 5.81–8.21%, and high intramuscular fat content was >8.21%.
[c] Statistical significance was *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, non-significant.

Furans are typically generated as auto-oxidation products during heating processes and are responsible of broiled and roasted meat odors (Flores et al., 1997).

4.3. Influence of HPP treatment on volatile compounds

HPP treatment could promote radical formation and lipid oxidation reactions in dry-cured ham (Andrés et al., 2004; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010), which could lead to changes in the volatile profile and affect product quality and acceptability. In the present study, the levels of alcohols, alkanes, esters, ketones, benzene compounds and sulfur compounds were significantly influenced in HPP-treated Serrano ham, after the 5-month refrigeration period. In Iberian ham, treatment at 600 MPa followed by a 30-day period at 4 °C under 12 h light/dark cycles, significantly increased the levels of linear aldehydes, considered as indicators of lipid oxidation, and the perception of rancid odor in the sensory analysis (Fuentes et al., 2010). In Serrano ham subjected to 600 MPa for 10 min at 21 °C, there was no significant effect of HPP on the levels of linear aldehydes and only the volatile compounds coming from microbial metabolism were influenced by HPP (Martínez-Onandi et al., 2016). No enhancement of lipid oxidation in sliced Serrano ham treated at 400 MPa for 10 min at 12 °C was observed after 3 days of refrigerated storage and most of the volatile compounds affected by HPP presumably derived from mold growth and metabolism (Rivas-Cañedo et al., 2009). In dry-cured pork loin subjected to 300, 350 or 400 MPa for 10 min at 20 °C and stored at 4 °C in the dark for 45 days, none of the aldehydes were affected by HPP (Campus, Flores, Martínez, & Toldrá, 2008). According to these authors, 2-pentanone, also coming from lipid oxidation, increased in samples treated at 400 MPa, but its level decreased during refrigerated storage. However, the effect of HPP was moderate on the levels of the volatile compounds coming from Maillard and Strecker reactions, with a decrease of trimethylpyrazine levels in the samples treated at pressures below 400 MPa (Campus et al., 2008).

In the present study, HPP of Serrano ham significantly affected the levels of 8 alcohols, 2 alkanes, 2 esters, 4 ketones, 1 benzene compound

Table 6
Levels[a] of the volatile compounds significantly influenced by high pressure processing (HPP) of Serrano ham after refrigerated storage for 5 months at 4 °C.

Compound	Untreated hams ($n = 30$)	HPP-treated hams (n = 30)	P^b
1-Octanol	1.57	2.63	***
2-Butanol	75.55	46.78	**
2-Pentanol	466.77	288.92	*
2-Hexanol	53.75	25.63	*
2-Heptanol	85.34	33.46	**
3-Methyl-2-butanol	10.69	7.16	**
1-Penten-3-ol	103.26	128.76	**
1-Octen-3-ol	78.10	98.97	*
Hexane	55.83	103.96	*
Dodecane	97.95	179.60	*
Ethyl acetate	69.63	44.36	*
Ethyl butanoate	17.12	9.89	*
2-Propanone	423.77	669.47	***
2-Butanone	341.00	470.40	***
2-Pentanone	488.42	994.78	***
5-Methyl-3-heptanone	6.89	8.95	**
3-Phenyl-2-propenal	1.94	2.31	*
Methanethiol	29.07	50.67	***
Sulfur dioxide	5.20	11.00	***
Dimethyl sulfide	15.40	10.97	*
Dimethyl disulfide	177.41	69.77	***

[a] Levels of volatile compounds are expressed as the sums of the abundance of characteristic ions, multiplied by 10^{-5}. Ham samples were analyzed in triplicate.
[b] Statistical significance was *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

and 4 sulfur compounds after 5 months at 4 °C. One linear alcohol (1-octanol), 2 secondary alcohols (1-penten-2-ol and 1-octen-3-ol), 2 alkanes (hexane and dodecane), 4 ketones (2-propanone, 2-butanone, 2-pentanone and 5-methyl-3-heptanone), 1 benzene compound (3-phenyl-2-propenal) and 2 sulfur compounds (methanethiol and sulfur dioxide) were significantly higher in HPP-treated ham than in untreated ham. In a previous study (Martínez-Onandi et al., 2016), the levels of ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, dimethyl disulfide and dimethyl trisulfide were higher in untreated samples while the levels of methanethiol and sulfur dioxide were higher in HPP-treated samples. Aldehydes, alcohols, ketones and esters come from oxidation reactions of fatty acids while sulfur compounds mainly derive from the sulfur containing amino acids via Strecker degradations to thiols (Toldrá & Flores, 1998). Microorganisms might contribute to the formation of volatile compounds in ham through the β-oxidation of fatty acids by molds or through amino acid catabolism by bacteria, but the physicochemical conditions present in ham are not favorable for microbial growth and metabolism (Rivas-Cañedo et al., 2009). In the present work, total microbial counts in individual hams remained below 6 log cfu/g, with mean counts for the 30 untreated hams below 4 log cfu/g (unpublished data). These low populations clearly limit microbial contribution to biochemical changes. However, the fact that microorganisms can act in Serrano ham during long ripening periods at temperatures close to 20 °C should not be neglected. During the 5-month refrigeration period, reactions involved in lipid oxidation were apparently promoted in HPP-treated ham while microbial metabolism seemed to be hindered by HPP.

HPP of meat products does not only affect the reactions involved in lipid oxidation, but it can also change the activity of enzymes by modifying their structure and those of their substrates (Cheftel & Culioli, 1997). The effect of HPP treatment on the oxidative stability of sliced vacuum-packaged dry-cured ham depended on the pressure level applied (Clariana et al., 2011; Clariana, Guerrero, Sárraga, & Garcia-Regueiro, 2012). According to these authors, HPP at 400 MPa increased superoxide dismutase activity and showed no effect on catalase and glutathione peroxidase activities whereas HPP at 600 MPa had no effect on any of the antioxidant enzyme activities and HPP at 900 MPa caused a decrease in superoxide dismutase and glutathione peroxidase activities and had no effect on catalase activity. These facts could influence the volatile profile of HPP-treated dry-cured ham and justify the variable results obtained by different authors.

5. Conclusions

Both physicochemical parameters and HPP treatment of Serrano ham influenced its volatile profile after 5 months of refrigerated storage. Intramuscular fat content was the physicochemical parameter affecting the highest number of volatile compounds (38 compounds) in untreated ham while a_w, salt content and salt-in-lean ratio only affected 4, 4 and 5 compounds, respectively. Ham of high intramuscular fat content showed higher levels of volatile compounds coming from lipid oxidation while ham of low fat content had higher levels of volatile compounds which could derive from microbial metabolism. It seems advisable not to keep Serrano ham of low intramuscular fat content for prolonged refrigerated storage periods, taking into account the enhanced formation of pyrazine and pyridine in this type of ham. HPP treatment affected 21 volatile compounds, resulting in higher levels of alkanes and ketones and lower levels of esters and secondary alcohols. Pressure-induced changes in the volatile profile of Serrano ham might affect its odor and aroma characteristics after 5 months of refrigerated storage.

Acknowledgments

The authors wish to thank E. Fulladosa, P. Gou and J. Arnau (IRTA, Monells, Spain) for the selection and processing of hams. Funding of project RTA2010-00029-C04-04 (Spanish Ministry of Science and Innovation) which supported the experimental work and funding of N. Martínez-Onandi Ph. D. grant by INIA are acknowledged.

References

Andrés, A. I., Cava, R., Ventanas, J., Muriel, E., & Ruiz, J. (2004). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, *84*, 375–381.
Andrés, A. I., Adamsen, C. E., Møller, J. K. S., Ruiz, J., & Skibsted, L. H. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research and Technology*, *222*, 486–491.
Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2007). Effect of salt content and processing conditions on volatile compounds formation throughout the ripening of Iberian ham. *European Food Research and Technology*, *225*, 677–684.
AOAC (2000). *Official methods of analysis* (17th ed.). Gaithersburg, Maryland, USA: Association of Official Analytical Chemists.
Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., & Mangia, A. (1992). Flavor compounds of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *40*, 2389–2394.
Berdagué, J. L., Denoyer, C., Le Quéré, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *39*, 1257–1261.
Campus, M., Flores, M., Martinez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, *80*, 1174–1181.
Carrapiso, A. I., & García, C. (2004). Iberian ham headspace: Odorants of intermuscular fat and differences with lean. *Journal of the Science of Food and Agriculture*, *84*, 2047–2051.
Carrapiso, A. I., Ventanas, J., & García, C. (2002). Characterization of the most odor-active compounds of Iberian ham headspace. *Journal of Agricultural and Food Chemistry*, *50*, 1996–2000.
Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, M. R. (2009). Effect of pressure and holding time on color, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigeration storage. *Innovative Food Science & Emerging Technologies*, *10*, 76–81.
Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat. *Meat Science*, *46*, 211–236.
Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, A., & Garcia-Regueiro, J. A. (2011). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Science & Emerging Technologies*, *12*, 456–465.
Clariana, M., Guerrero, L., Sárraga, C., & Garcia-Regueiro, J. A. (2012). Effects of high pressure application (400 and 900 MPa) and refrigerated storage time on the oxidative stability of sliced skin vacuum packed dry-cured ham. *Meat Science*, *50*, 323–329.
Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry*, *45*, 2178–2186.

Flores, M., Gianelli, M. P., Pérez-Juan, M., & Toldrá, F. (2007). Headspace concentration of selected dry-cured aroma compounds in model systems as affected by curing agents. *Food Chemistry, 102*, 488–493.
Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method from the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.
Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatics pressure. *Meat Science, 85*, 506–514.
García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry, 41*, 23–32.
Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science & Emerging Technologies, 5*, 451–457.
Grasso, S., Brunton, N. P., Lyng, J. G., Lalor, F., & Monahan, F. J. (2014). Healthy processed meat products - Regulatory, reformulation and consumer challenges. *Trends in Food Science & Technology, 39*, 4–17.
Hinrichsen, L. L., & Pedersen, S. B. (1995). Relationship among flavor, volatile compounds, chemical-changes, and microflora in Italian-type dry-cured ham during processing. *Journal of Agricultural and Food Chemistry, 43*, 2932–2940.
Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology, 110*, 8–18.
Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., & Picon, A. (2016). Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. *Meat Science, 111*, 130–138.
Min, D. B. S., Ina, K., Peterson, R. J., & Chang, S. S. (1977). The alkylbenzenes in roast beef. *Journal of Food Science, 42*, 503–505.
Motilva, M. J., & Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift für Lebensmittel-Untersuchung und - Forschung, 196*, 228–232.
Narváez-Rivas, M., Vicario, I. M., Constante, E. G., & León-Camacho, M. (2007). Changes in the concentrations of free fatty acid, monoacylglycerol, and diacylglycerol in the subcutaneous fat of Iberian ham during the dry-curing process. *Journal of Agricultural and Food Chemistry, 55*, 10953–10961.
Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: A review. *Grasas y Aceites, 63*, 432–454.
Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry, 55*, 1923–1931.
Rivas-Cañedo, A., Fernández-García, E., & Núñez, M. (2009). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing; effect of the packaging material. *Meat Science, 82*, 321–328.
Ruiz, J., Ventanas, J., & Cava, R. (2001). New device for direct extraction of volatile in solid samples using SPME. *Journal of Agricultural and Food Chemistry, 49*, 5115–5121.
Ruiz, J., García, C., Muriel, E., Andrés, A. I., & Ventanas, J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science, 61*, 347–354.
Sabio, E., Vidal-Aragón, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from South European countries. *Food Chemistry, 61*, 493–503.
Sánchez-Peña, C. M., Luna, G., García-González, D. L., & Aparicio, R. (2005). Characterization of French and Spanish dry-cured hams: Influence of the volatile from the muscles and the subcutaneous fat quantified by SPME-GC. *Meat Science, 69*, 635–645.
Shahidi, F., Rubin, L. J., & D'Souza, L. A. (1986). Meat flavor volatiles - A review of the composition, techniques of analysis, and sensory evaluation. *Critical Reviews in Food Science and Nutrition, 24*, 141–243.
Spinnler, H. E., Berger, C., Lapadatescu, C., & Bonnarme, P. (2001). Production of sulfur compounds by several yeasts of technological interest for cheese ripening. *International Dairy Journal, 11*, 245–252.
Stahnke, L. H. (1994). Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Science, 38*, 39–53.
Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels - Part II. Volatile components. *Meat Science, 41*, 193–209.
Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A., & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science, 58*, 85–91.
Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science, 49*, S101–S110.
Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. *Trends in Food Science & Technology, 17*, 164–168.
Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition, 38*, 331–352.
Toldrá, F., Rico, E., & Flores, J. (1993). Cathepsin B, D, H and L activities in the processing of dry-cured ham. *Journal of the Science of Food and Agriculture, 62*, 157–161.
Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Journal of Food Science, 57*, 813–815.
Ventanas, S., Estevez, M., Andrés, A. I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME-DED. *Meat Science, 79*, 172–180.

Meat Science 125 (2017) 152

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Corrigendum

Corrigendum to "Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage" [Meat Science volume (2016) 101–108]

N. Martínez-Onandi, A. Rivas-Cañedo, A. Picon, M. Nuñez *

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, Madrid 28040, Spain

The authors would like to correct the following errors in their article:

Martínez-Onandi, N., Rivas-Cañedo, A., Picon, A., & Nuñez, M. (2016). Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage. Meat Science 122, 101–108.
http://dx.doi.org/10.1016/j.meatsci.2016.07.027

1. In page 102, 2nd column, 4th paragraph, the first two sentences should be read as:
NaCl concentrations of hams ranged from 3.21% to 6.77%, with a mean value of 4.41% NaCl on total weight (SD, 0.83). They were grouped into 10 hams of low (<4.00%), 12 of medium (4.00–4.82) and 8 of high (>4.82) salt content.

2. In page 102, 2nd column, 5th paragraph, the first two sentences should be read as:
The salt-in-lean ratio of hams ranged from 0.035 to 0.069, with a mean value of 0.047 (SD, 0.009). They were grouped into 10 hams of low (<0.043), 12 of medium (0.043–0.052) and 8 of high (>0.052) salt-in-lean ratio.

3. In page 104, Table 3, the "b" footnote should be read as:
[b]Low salt (NaCl) content was <4.00%, medium salt content was within the range 4.00–4.82%, and high salt content was >4.82%.

4. In page 105, Table 4, the "b" footnote should be read as:
[b]Low S/L ratio was <0.043, medium S/L ratio was within the range 0.043–0.052, and high S/L ratio was >0.052.
The authors would like to apologise for any inconvenience caused.

DOI of original article: http://dx.doi.org/10.1016/j.meatsci.2016.07.027.
* Corresponding author.
E-mail address: nunez@inia.es (M. Nuñez).

http://dx.doi.org/10.1016/j.meatsci.2016.11.019

4. Microbiota of high-pressure-processed Serrano ham investigated by culture-dependent and culture-independent methods

Artículo publicado en *International Journal of Food Microbiology* 241 (2017) 298-307

International Journal of Food Microbiology 241 (2017) 298–307

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Microbiota of high-pressure-processed Serrano ham investigated by culture-dependent and culture-independent methods

N. Martínez-Onandi [a,b], A. Castioni [b], E. San Martín [a], A. Rivas-Cañedo [a], M. Nuñez [a], S. Torriani [b], A. Picon [a,*]

[a] Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, Madrid 28040, Spain
[b] Dipartimento di Biotecnologie, Università degli Studi di Verona, 37134 Verona, Italy

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form 28 October 2016
Accepted 2 November 2016
Available online 05 November 2016

Keywords:
Serrano ham microbiota
Salt content
Intramuscular fat
High pressure processing
DGGE

ABSTRACT

The microbiota of Serrano dry-cured ham of different chemical composition, subjected or not to high-pressure processing (HPP), was investigated using culture-dependent and culture-independent methods. Microbial counts were submitted to analysis of variance with physicochemical parameters (a_w, NaCl concentration, salt-in-lean ratio and intramuscular fat content) or HPP as main effects. In untreated hams, physicochemical parameters significantly affected counts of aerobic mesophiles, psychrotrophs, and moulds and yeasts. NaCl concentration and fat content influenced the levels of four and three of the five studied microbial groups, respectively, whereas no influence of a_w was stated. The HPP treatment had a significant effect on counts of all investigated microbial groups. Culture-independent methods showed the presence of bacteria such as *Staphylococcus equorum*, *Staphylococcus succinus*, *Bacillus subtilis* and *Cellulosimicrobium* sp., moulds like *Penicillium commune*, *Aspergillus fumigatus*, *Sclerotinia sclerotiorum*, *Eurotium athecium* and *Moniliella mellis*, and yeasts like *Debaryomyces hansenii* and *Candida glucosophila*. Absence of *B. subtilis* bands and weaker bands of *E. athecium* were recorded for HPP-treated hams.
The higher microbial levels found in lean ham might result in a quicker deterioration. HPP treatment confirmed its suitability as a procedure to control spoilage microorganisms. DGGE did not seem to be sensitive enough to highlight changes caused by HPP treatment in the microbiota of ham, but contributed to the detection of microbial species not previously found in ham.

1. Introduction

Serrano ham is a Spanish dry-cured meat product highly appreciated worldwide, with an annual production exceeding 125 million kg. The particular sensory characteristics of Serrano ham are mostly due to the activity of muscle proteinases and lipases (Toldrá and Flores, 1998) throughout a ripening period which may last up to 18 months.

The manufacturing process of Serrano ham starts with a salting step, during which a microbial community primarily consisting in microorganisms present in the salt colonizes the ham surface (Cornejo et al., 1992). During Serrano ham ripening, the growth of most microorganisms in raw meat is limited by the environmental conditions they face (low a_w and pH in the product, and low relative humidity and low to medium temperatures in the cold room). Only microbial groups adapted to those adverse conditions, in particular to the low a_w values, will survive throughout ripening of Serrano ham. Some of those microorganisms are proteolytic and/or lipolytic and certain species can reduce nitrates to nitrites, thus contributing to the sensory properties of ham (Cordero and Zumalacárregui, 2000). According to these authors, *Micrococcaceae* was the most abundant microbial group in salt used in the manufacture of dry-cured ham. Gram-positive, catalase-positive cocci were identified as the predominant microorganisms in different types of dry-cured ham (Giolitti et al., 1971; Langlois and Kemp, 1974; Rodríguez et al., 1994). Most *Micrococcaceae* isolates from Iberian dry-cured ham belonged to the genus *Staphylococcus*, with *S. xylosus* as the predominant species, although a remarkable diversity of *Staphylococcus* and *Micrococcus* species throughout ripening was detected (Rodríguez et al., 1994). *Staphylococcus* and *Micrococcus* isolates from ham possess enzymatic activities such as nitrate reductase, catalase, lipases and proteinases (Giolitti et al., 1971) which could influence ham sensory characteristics.

Moulds and yeasts are one of the major microbial groups during dry-cured ham processing and may contribute to the development of flavour characteristics (Lücke, 1986). Moulds belonging to the genera *Eurotium*, *Penicillium* and *Trichoderma* and yeasts identified as *Debaryomyces marama* were isolated from surface samples of Iberian dry-cured ham (Monte et al., 1986). In another study, mould isolates from surface samples of Iberian dry-cured ham mostly belonged to the genera *Penicillium*, *Aspergillus* and *Eurotium*, although members of the genera *Aureobasidium*, *Cladosporium*, *Curvularia* and *Syncephalastrum* were also detected (Núñez et al., 1996a). Yeasts isolated from the

* Corresponding author.
E-mail address: apicon@inia.es (A. Picon).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.11.001
0168-1605/

same surface samples of Iberian dry-cured ham were mainly identified as *Candida zeylanoides* and *Debaryomyces hansenii*, with *C. blankii*, *C. intermedia*, *Pichia carsonii* and *Rhodotorula rubra* also found sporadically (Núñez et al., 1996b). Some of the *D. hansenii* isolates showed high proteolytic activity against raw pork myofibrillar proteins (Martín et al., 2001; Rodríguez et al., 1998).

Consumers demand high quality, tasty, healthy, natural, convenient and safe meat products with an extended shelf life. On top of that, meat products should contain less salt, fat, acid and chemical preservatives (Aymerich et al., 2008). Producers in the meat industry try to adapt to consumers' demand for healthier food products elaborating products with low salt and fat contents. In dry-cured ham, salt contributes to microbial stability by lowering a_w, enhances protein solubilization, affects proteolysis, lipolysis and lipid oxidation, improves product texture and directly contributes to flavour (Toldrá and Flores, 1998). A reduction in salt content may increase microbial risk and cause technological problems, resulting in more pronounced rancid, fatty and buttery aroma notes than those of hams of high salt content (Coutron-Gambotti et al., 1999).

Although whole dry-cured ham is considered a shelf-stable product due to its salt content (up to 8–10% on total weight) and water activity (a_w, usually below 0.90), the trend for convenient products has resulted in an increasing percentage of hams being deboned, sliced and vacuum packaged at the processing plants (Morales et al., 2006). All these post-process operations increase the risk of microbial cross-contamination by pathogens and spoilage microorganisms, which could affect the safety and compromise the shelf life of the product. The presence and survival of *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* in ham packages from different manufacturers has been reported (Ng et al., 1997). In a ready-to-eat product as Serrano ham, these pathogens, which are difficult to eradicate from processing plants, may pose a safety risk to consumers.

High-pressure processing (HPP), a non-thermal technology that causes destruction of microbial vegetative cells and inactivation of certain enzymes, with minor changes in the sensory characteristics of meat products (Garriga et al., 2004), is being increasingly used for the decontamination of ready-to-eat meat products (Aymerich et al., 2008). Food composition influences the effect of HPP on microorganisms. Low a_w values and/or high solute concentration exert a baroprotective effect and reduce the extent of bacterial inactivation induced by HPP (Patterson, 2005). A significant reduction of at least 2 log units for spoilage microorganisms in vacuum-packed sliced dry-cured ham was recorded after HPP at 600 MPa for 6 min (Garriga et al., 2004). Also, the effect of HPP at 450 MPa for 10 min on *Listeria monocytogenes* in Serrano and Iberian dry-cured hams was investigated (Morales et al., 2006), with differences in pathogen lethality between types of ham ascribed by the authors to NaCl concentration. HPP of foods may result in the appearance of sublethally injured survivors unable to grow on culture media, in particular when plated on selective agars. Non-culturable bacteria have been detected in HPP-treated blood sausage, goat milk curd and cooked ham using culture-independent molecular techniques (Campos et al., 2011; Diez et al., 2008; Han et al., 2011).

HPP of Serrano ham and its chemical composition are known to affect the formation of volatile compounds during ripening (Martínez-Onandi et al., 2016). However, to our knowledge, there is no available information on the effect of chemical composition on the microbiota of ham or how this chemical composition would modulate the effect of HPP on microorganisms. The objective of the present study was to investigate Serrano ham microbiota before and after HPP as well as the effect of ham chemical composition on the microbiota by means of culture-dependent and culture-independent methods.

2. Materials and methods

2.1. Selection and manufacture of Serrano hams

Serrano ham manufacture was carried out at the Institute of Food and Agricultural Research and Technology (IRTA, Monells, Spain). Thirty green hams were selected at commercial slaughterhouses from animals of different genotype in order to obtain a wide range of fat contents. Twenty one hams were from Large White x Landrace animals and nine hams from animals with a minimum of 50% Duroc breed. Fat content of entire hams was determined using a magnetic resonance sensor technology (JMP Ingenieros, Sotés, Spain). Homogeneous hams in terms of weight and pH were used in experiments. Average weight of hams was 11.77 ± 0.66 kg whereas the pH in the semimembranosus muscle at 24 h post-mortem ranged from 5.4 to 5.9. Hams were manually rubbed with the following mixture (per kg of raw ham): 10 g NaCl, 1.0 g dextrose, 0.5 g ascorbic acid, 0.15 g KNO_3 and 0.15 g $NaNO_2$. Afterwards, hams were individually salted with excess of salt at 3 ± 2 °C and 85 ± 5% RH for 0.6 to 1.5 days/kg of raw ham in order to obtain a wide range of salt contents. After salting, hams were washed with cold water, weighed and hung in a cold room at 3 °C and 75–80% RH to rest. Temperature was progressively increased up to 20 °C during ripening. The process was finished when a total weight loss of 36% was achieved.

2.2. Sampling and high pressure processing

The superficial part of the cushion was removed with a sterile knife. Afterwards, the whole cushion (mainly composed of the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* muscles) from each ham was extracted with another sterile knife and two slices (approximately 150 g) were obtained and individually vacuum-packaged. One of the slices was HPP-treated at 600 MPa for 6 min at 21 °C (pressure build up time, 2.5 min; pressure release time <2 s) in a 120 L capacity Wave 6000 equipment (Hiperbaric, Burgos, Spain) at IRTA, whereas the untreated slice served as control. Ham slices were held at 4 °C prior to analysis, which was carried out within 3 days of HPP.

2.3. Physicochemical determinations

Water activity (a_w) was determined using an AquaLab Series 3-equipment (Decagon Devices, Inc., Pullman, WA, USA). Chloride content was analysed by the Volhard method (AOAC, 2000) and intramuscular fat content by a previously described method (Folch et al., 1957). All determinations were performed in triplicate.

2.4. Microbiological analysis

Representative ham samples (10 g) were aseptically taken and homogenized with 90 mL of a sterile saline peptone solution (Maximum recovery diluent, Biolife, Milano, Italy) in a Colworth Stomacher 400 (A. J. Seward Ltd., London, UK) for 3 min. Serial dilutions were prepared and plated in duplicate onto appropriate culture media. Aerobic mesophiles were enumerated on Plate Count Agar (PCA, Biolife) after incubation for 48 h at 30 °C, psychrotrophs on PCA after incubation for 7 days at 8 °C, *Enterobacteriaceae* in double-layered plates of Violet Red Bile Glucose Agar (VRBG, Biolife) after incubation for 24 h at 37 °C, lactic acid bacteria (LAB) on MRS Agar (Biolife) after anaerobic incubation for 72 h at 30 °C, enterococci on Kanamycin Aesculin Azide Agar (KAA, Oxoid, Basingstoke, Hampshire, UK), after incubation for 24 h at 37 °C, *Micrococcaceae* on Mannitol Salt Agar (MSA, Oxoid) after incubation for 36 h at 37 °C, coagulase-positive staphylococci on Baird-Parker agar with rabbit plasma fibrinogen (RPF) Supplement II (BP + RPF, Biolife) after incubation for 24 h at 37 °C, and moulds and yeasts on Sabouraud Dextrose Agar (SDA, Oxoid) after incubation for 5 days at 25 °C. Microbial counts were expressed as log cfu per gram of ham. Presence of *L. monocytogenes* was investigated in 25 g of each

ham by pre-enrichment in Listeria UVMI enrichment broth (Biolife) for 24 h at 30 °C and enrichment in Fraser broth (Biolife) for 24 h at 37 °C, followed by plating on CHROMagar Listeria (Scharlau, Barcelona, Spain). Presence of *Salmonella* spp. was investigated in 25 g samples by pre-enrichment in buffered peptone water (Oxoid) for 20 h at 37 °C and enrichment in two selective media (Rappaport Vasiliadis Soy broth (RVS, Biolife) for 24 h at 42 °C and Muller-Kauffmann Tetrathionate-novobiocin (MKTTn, Oxoid) for 24 h at 37 °C), followed by plating on Xylose Lysine Desoxycholate agar (XLD, Oxoid) and Brilliance Salmonella agar (BSA, Oxoid).

2.5. Isolation of microbial DNA from Serrano ham samples

Ham homogenates (5 g of ham and 10 mL of a sterile saline peptone solution) were prepared and aliquots stored at −20 °C. Homogenate samples (1 mL) were placed in 2 mL screw-cap tubes containing 0.3 g of glass beads, 400 μL of a 50 mg/mL lysozyme solution (Sigma-Aldrich Co., St Louis, MI, USA) and 25 μL of a 10 U/mL lyticase solution (Sigma-Aldrich Co.). Tubes were incubated at 37 °C for 30 min, cooled to room temperature and subjected to a mechanical disruption treatment in a mini-bead beater apparatus (Biospec Products Inc., Bartlesville, Oklahoma, USA) by alternating 1 min at maximum speed with 1 min on ice, 3 times. Total microbial DNA was isolated with the Wizard Genomic DNA Purification Kit A1125 (Promega, Fichburg, WI, USA) following manufacturer's recommendations.

2.6. PCR amplification and DGGE analyses

Total DNA from ham samples was used as template material to amplify the V3 region of the bacterial 16S rRNA gene by performing a PCR with the universal primers HDA1-GC and HDA2 (Walter et al., 2000). The reaction mixture (40 μL) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of dNTP, 1 μM of each primer, template DNA at a final concentration of 100 ng/mL and 0.25 U of *Taq* polymerase (Promega). PCR amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Fostercity, CA, USA), under the following conditions: 94 °C for 4 min, 30 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, and a final step of 72 °C for 8 min. For eukaryotic organisms, the 5′-end region of the 18S rRNA gene was amplified by PCR using primers NS1 (White et al., 1990) and GCFung (May et al., 2001). The reaction mixture (50 μL) contained 10 mM Tris-HCl (pH 8.3), 50 KCl mM, 1 mM $MgCl_2$, 0.2 mM of dNTP, 0.3 μM of each primer, template DNA at a final concentration of 100 ng/mL and 0.8 U of *Taq* polymerase (Promega). PCR amplification was performed according to a previously described method (Duong et al., 2006). The D1 domain of the 26S rRNA gene was amplified by PCR using the universal primers NL1-GC and LS2 (Cocolin et al., 2000). The reaction was performed in a final volume of 50 μL containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.25 mM $MgCl_2$, 0.2 mM of dNTP, 0.5 μM of each primer, template DNA at a final concentration of 100 ng/mL and 2 U of *Taq* polymerase (Promega). PCR amplification was performed under the following conditions: 95 °C for 5 min, 36 cycles of 95 °C for 1 min, 52 °C for 2 min and 72 °C for 2 min, and a final step of 72 °C for 7 min. All PCR products were analysed by electrophoresis in 1.5% (w/v) agarose gel prepared in 1× Tris-Acetate-EDTA (TAE) buffer and the GeneRuler DNA ladder mix (ThermoFisher scientific, Waltham, MA, USA) was used as molecular size marker.

The obtained PCR products were subjected to DGGE analysis in a DCode Universal Mutation Detection System apparatus (Bio-Rad, Richmond, CA, USA). Thirty microliters of PCR product plus 30 μL of loading buffer were directly applied onto 8% (w/v) polyacrylamide gels with formamide-urea (Bio-Rad) denaturing ranges of 30% to 50% for 16S rRNA and 26S rRNA amplicons, and 20% to 40% for 18S rRNA amplicons. Gels were electrophoresed in 1× TAE buffer at 130 V and 50 °C for 17 h, stained with SYBR green for 10 min, rinsed in distilled water and photographed under UV illumination.

2.7. Identification of DGGE bands

Selected DGGE bands were excised from gels with sterile scalpel blades. The DNA was allowed to diffuse in 50 μL TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). One microliter of the eluted DNA was used as template for reamplification using the original pair of primers without the GC-clamp. Bacterial PCR products were loaded onto agarose gels and purified using the NucleoSpin Gel and PCR Clean-up extraction kit (Macherey-Nagel GmbH, Düren, Germany). Eukaryotic PCR products were purified by adding 150 μL TE, 19.5 μL 3 M sodium acetate and 429 μL of cool 70% ethanol. Mixtures were held at −80 °C for 1 h. Tubes were then centrifuged at 14,000 ×g and 4 °C for 10 min. Pellets were washed with 400 μL of cool 70% ethanol, vacuum dried at room temperature and resuspended in 50 μL of sterile water. Purified amplicons were sequenced at a commercial sequencing facility (GATC biotech, Cologne, Germany). The obtained partial 18S and 26S rDNA sequences were analysed with the BLASTN program provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al., 1997), while the obtained partial 16S rDNA sequences were analysed using the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012) in order to determine their closest relatives.

2.8. Statistical analysis

Microbial count data were analysed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was carried out with each of the physicochemical parameters (a_w, NaCl concentration, intramuscular fat content, salt-in-lean ratio) or HPP as main effects. Hams were divided in three groups (low, medium and high values) for each physicochemical parameter. The mean ± 0.5 standard deviation (SD) criterion was used to establish these three groups. Means were compared by Tukey's test, with the significance assigned at $P < 0.05$.

3. Results

3.1. Effect of ham chemical composition on microbial counts

The a_w values of Serrano ham ranged from 0.833 to 0.883 (mean, 0.859; SD, 0.013). Hams were grouped into 11 low a_w (<0.853) hams, 9 medium a_w (0.853–0.864) hams and 10 high a_w (>0.864) hams. Values of a_w did not significantly influence the counts of any of the microbial groups in either untreated or HPP-treated ham (Table 1).

NaCl concentration of hams ranged from 2.87% to 7.91% (mean, 5.49%; SD, 1.30%). Hams were grouped into 7 hams of low (<4.83%), 13 of medium (4.83–6.13%) and 10 of high (>6.13%) NaCl content. The concentration of NaCl significantly influenced counts of aerobic mesophiles, psychrotrophs, lactic acid bacteria, and moulds and yeasts in untreated ham (Table 2). High NaCl concentration hams had the highest levels of aerobic mesophiles, psychrotrophs, and moulds and yeasts while medium NaCl concentration hams had the highest levels of lactic acid bacteria. In HPP-treated hams, no significant effect of NaCl concentration on the counts of any microbial group was recorded.

Salt-in-lean ratio of hams ranged from 0.033 to 0.081 (mean, 0.058; SD, 0.013). Hams were grouped into 7 hams of low (<0.050), 13 of medium (0.050–0.066) and 10 of high (>0.066) salt-in-lean-ratio. The salt-in-lean ratio significantly influenced the levels of aerobic mesophiles, psychrotrophs, and moulds and yeasts in untreated ham (Table 3). High salt-in-lean ratio hams had higher levels of these three microbial groups than medium or low salt-in-lean ratio hams. Salt-in-lean ratio did not significantly influence the levels of any microbial group in HPP-treated hams.

Intramuscular fat content of hams ranged from 1.83% to 14.03% (mean, 5.28%; SD, 3.45%). Hams were grouped into 8 hams of low (<3.56%), 11 of medium (3.56–6.99%) and 11 of high (>6.99%) intramuscular fat content. Fat content significantly influenced the levels

Table 1
Counts[1] of the main microbial groups in untreated and HPP-treated Serrano ham as influenced by a_w.[2]

	Untreated ham				HPP-treated ham			
Microbial group	Low a_w	Medium a_w	High a_w	P[3]	Low a_w	Medium a_w	High a_w	P[3]
Aerobic mesophiles	3.17 ± 1.40[a]	2.94 ± 1.02[a]	3.16 ± 0.87[a]	ns	1.29 ± 0.38[a]	1.57 ± 0.64[a]	1.53 ± 1.05[a]	ns
Psychrotrophs	3.10 ± 1.84[a]	2.78 ± 1.59[a]	3.15 ± 0.96[a]	ns	1.07 ± 0.23[a]	1.34 ± 0.53[a]	1.51 ± 1.02[a]	ns
Lactic acid bacteria	1.63 ± 0.78[a]	1.55 ± 0.55[a]	1.46 ± 0.73[a]	ns	1.00 ± 0.00[a]	1.26 ± 0.54[a]	1.06 ± 0.22[a]	ns
Micrococcaceae	2.19 ± 1.40[a]	2.23 ± 0.62[a]	2.59 ± 1.03[a]	ns	1.07 ± 0.23[a]	1.15 ± 0.48[a]	1.35 ± 0.97[a]	ns
Moulds and yeasts	2.77 ± 1.87[a]	2.92 ± 1.24[a]	3.18 ± 0.57[a]	ns	1.00 ± 0.00[a]	1.08 ± 0.25[a]	1.00 ± 0.00[a]	ns

[a,b]Means in the same row followed by different superscripts differ ($P < 0.05$).
[1] Counts are expressed in log cfu g^{-1} (mean ± SD of duplicate determinations).
[2] Low a_w was <0.853, medium a_w was within the range 0.853–0.864, and high a_w was >0.864.
[3] Statistical significance in ANOVA: ns, non-significant.

of aerobic mesophiles, psychrotrophs, and moulds and yeasts in untreated ham (Table 4). Low fat content hams had higher levels of aerobic mesophiles, psychrotrophs, and moulds and yeasts than medium or high fat content hams. Intramuscular fat content did not significantly influence the levels of any microbial group in HPP-treated hams.

3.2. Effect of HPP on microbial counts

Counts of the different microbial groups in untreated and HPP-treated hams are shown in Table 5. All the analysed microbial groups were significantly ($P < 0.05$) affected by HPP. In untreated ham samples, average counts of 3.09, 3.01, 1.55, 2.35 and 2.97 log cfu g^{-1} were recorded for aerobic mesophiles, psychrotrophs, lactic acid bacteria, *Micrococcaceae* and moulds and yeasts, respectively. After HPP, reductions of 1.63, 1.71, 0.44, 1.15 and 1.95 log cfu g^{-1} were recorded for counts of aerobic mesophiles, psychrotrophs, lactic acid bacteria, *Micrococcaceae* and moulds and yeasts, respectively. Neither *L. monocytogenes* nor *Salmonella* spp. were detected after enrichment in any of the untreated or HPP-treated Serrano ham samples (25 g).

3.3. DGGE analyses of ham samples

Fifteen untreated ham samples (those with the highest counts of aerobic mesophiles) and the corresponding HPP-treated ham samples were selected to be analysed by DGGE. The DGGE profiles of the PCR amplicons belonging to the V3 region of bacterial 16S rRNA gene are shown in Fig. 1. Thirty-seven bands were cut, reamplified, purified and sequenced but only 10 of them gave readable sequences (Table 6). Two of the retrieved sequences corresponded to portions of the 16S rRNA gene of porcine mitochondria. Members of the genera *Staphylococcus* (*S. equorum* and *S. succinus*), *Bacillus* (*B. subtilis*) and *Cellulosimicrobium* were among the microbiota of untreated Serrano ham samples. However, bands of *B. subtilis* were not found in HPP ham samples.

The DGGE profiles of the PCR amplicons belonging to the 5′-end region of 18S rRNA gene are shown on Fig. 2. Sixteen bands were cut, reamplified, purified and sequenced and 12 of them gave readable sequences (Table 7). The DGGE profiles of the PCR amplicons belonging to the D1 domain of 26S rRNA gene are shown on Fig. 3. Seven bands were cut, reamplified, purified and sequenced, but only 3 of them gave readable sequences (Table 8). The mould population appeared to be mainly composed of *Penicillium commune*, *Aspergillus fumigatus*, *Sclerotinia sclerotiorum*, *Eurotium athecium* and *Moniliella mellis*. The presence of *Penicillium chrysogenum* was observed in only one untreated ham sample. Most of the bands remained unaltered after HPP of hams, although bands of *E. athecium* were weaker in HPP ham samples than in untreated samples. The species *Debaryomyces hansenii* and *Candida glucosophila* were the only identified members of the yeast community of Serrano ham. HPP of hams seemed to have little effect on the bands corresponding to the yeast population.

4. Discussion

The effect of physicochemical parameters and HPP on the counts of the main microbial groups present in Serrano ham was elucidated in the present study. Microbial levels in the interior of Serrano ham were generally low, not exceeding 6 log cfu g^{-1} in any of the untreated individual ham samples. Average microbial counts in untreated Serrano ham were close to 3 log cfu g^{-1} for aerobic mesophiles, psychrotrophs, and moulds and yeasts while lactic acid bacteria and *Micrococcaceae* only reached 1.55 log cfu g^{-1} and 2.35 log cfu g^{-1}, respectively. Those levels were similar to microbial counts in samples from the cushion of Parma ham (Hinrichsen and Pedersen, 1995) and to the counts recorded in raw hams of Italian type after 4 months at 8 °C, but one log unit higher than those recorded after 6 months at 4 °C (Giolitti et al., 1971). Higher counts of aerobic mesophiles (4.79 log cfu g^{-1}) and lactic acid bacteria (3.33 log cfu g^{-1}) were found in vacuum-packaged Serrano dry-cured ham slices (Garriga et al., 2004), what could be partly explained by microbial contamination of ham during slicing and packaging. Higher microbial counts were also reported for Serrano ham by Blesa et al. (2008), who found mesophilic aerobic counts ranging from 3 to 8 log cfu g^{-1} and lactic acid bacteria counts ranging from 2 to 6 log cfu g^{-1}, depending on sample location and time of post-salting. Some studies on the enzymatic activities of microorganisms isolated from dry-cured ham suggest a relationship between the secondary metabolism of those microorganisms and the development of dry-cured

Table 2
Counts[1] of the main microbial groups in untreated and HPP-treated Serrano ham as influenced by NaCl concentration.[2]

	Untreated ham				HPP-treated ham			
Microbial group	Low NaCl	Medium NaCl	High NaCl	P[3]	Low NaCl	Medium NaCl	High NaCl	P[3]
Aerobic mesophiles	2.26 ± 1.06[b]	3.18 ± 0.73[ab]	4.29 ± 1.58[a]	**	1.53 ± 0.57[a]	1.15 ± 0.34[a]	1.93 ± 1.21[a]	ns
Psychrotrophs	1.96 ± 1.04[b]	3.10 ± 0.99[b]	4.58 ± 1.31[a]	***	1.28 ± 0.64[a]	1.12 ± 0.29[a]	1.71 ± 1.13[a]	ns
Lactic acid bacteria	1.40 ± 0.48[ab]	1.91 ± 0.82[a]	1.17 ± 0.44[b]	*	1.22 ± 0.50[a]	1.06 ± 0.21[a]	1.00 ± 0.00[a]	ns
Micrococcaceae	1.89 ± 0.79[a]	2.47 ± 1.13[a]	2.88 ± 1.13[a]	ns	1.00 ± 0.00[a]	1.12 ± 0.29[a]	1.66 ± 1.24[a]	ns
Moulds and yeasts	2.32 ± 1.02[b]	2.85 ± 0.98[b]	4.21 ± 1.39[a]	**	1.00 ± 0.00[a]	1.06 ± 0.19[a]	1.00 ± 0.00[a]	ns

[a,b]Means in the same row followed by different superscripts differ ($P < 0.05$).
[1] Counts are expressed in log cfu g^{-1} (mean ± SD of duplicate determinations).
[2] Low NaCl concentration was <4.83%, medium NaCl concentration was within the range 4.83–6.13%, and high NaCl concentration was >6.13%.
[3] Statistical significance in ANOVA: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3
Counts[1] of the main microbial groups in untreated and HPP-treated Serrano ham as influenced by the salt-in-lean (S/L) ratio.[2]

	Untreated ham				HPP-treated ham			
Microbial group	Low S/L	Medium S/L	High S/L	P^3	Low S/L	Medium S/L	High S/L	P^3
Aerobic mesophiles	2.31 ± 1.14^{b}	2.97 ± 0.86^{b}	4.29 ± 1.57^{a}	**	1.53 ± 0.64^{a}	1.21 ± 0.37^{a}	1.93 ± 1.21^{a}	ns
Psychrotrophs	1.90 ± 1.08^{b}	2.90 ± 1.04^{b}	4.58 ± 1.31^{a}	***	1.38 ± 0.73^{a}	1.09 ± 0.26^{a}	1.70 ± 1.13^{a}	ns
Lactic acid bacteria	1.45 ± 0.51^{ab}	1.77 ± 0.79^{a}	1.16 ± 0.44^{b}	ns	1.30 ± 0.56^{a}	1.05 ± 0.19^{a}	1.00 ± 0.00^{a}	ns
Micrococcaceae	2.06 ± 0.76^{a}	2.26 ± 1.12^{a}	2.84 ± 1.12^{a}	ns	1.00 ± 0.00^{a}	1.09 ± 0.26^{a}	1.65 ± 1.23^{a}	ns
Moulds and yeasts	2.45 ± 1.02^{b}	2.67 ± 1.03^{b}	4.20 ± 1.39^{a}	***	1.00 ± 0.00^{a}	1.05 ± 0.19^{a}	1.00 ± 0.00^{a}	ns

^{a,b}Means in the same row followed by different superscripts differ ($P < 0.05$).
[1] Counts are expressed in log cfu g^{-1} (mean ± SD of duplicate determinations).
[2] Low salt-in-lean ratio was <0.050, medium salt-in-lean ratio was within the range 0.050–0.066, and high salt-in-lean ratio was >0.066.
[3] Statistical significance in ANOVA: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ham sensory characteristics (Córdoba et al., 1994, Rodríguez et al., 1994). In fact, differences in ham chemical composition affected some volatile compounds presumably of microbial origin (Martínez-Onandi et al., 2016) and might as well affect the growth and metabolism of ham microbiota. In the present study, NaCl concentration, salt-in-lean ratio and intramuscular fat content exerted significant effects on counts of aerobic mesophiles, psychrotrophs, and moulds and yeasts whereas no effect of a_w on microbial counts was observed.

High a_w values generally enhance microbial growth and metabolism. However, some xerophile species of moulds and yeasts are able to grow abundantly on low a_w meat products (Rubio et al., 2007). The resistance of microorganisms to HPP increased with decreasing a_w, as shown in a study on the inactivation of *Staphylococcus aureus* and lactic acid bacteria after HPP at 600 MPa for 6 min in marinated beef, cooked ham and dry-cured ham, meat products with different a_w (Hugas et al., 2002). When *L. monocytogenes* resistance to HPP in Serrano and Iberian ham was compared, the lower a_w of Serrano ham seemed to protect the pathogen during treatment, resulting in higher survival immediately after treatment, but thereafter the lower a_w of Serrano ham was probably less favourable for the recovery of injured cells and the viability of survivors (Morales et al., 2006). In the present study, counts of microbial groups in untreated or HPP-treated hams were not significantly influenced by a_w values. This result is in apparent contradiction with the fact that some volatile compounds of microbial origin (ethanol, 2-propanol, 1-methoxy-2-propanol, ethyl acetate and dimethyl sulphide) were at higher levels in high a_w hams than in medium or low a_w hams (Martínez-Onandi et al., 2016). However, changes in microbial metabolic activity at different a_w values might partly explain the differences in volatile compound levels.

Salt, added at the beginning of ham manufacturing process, inhibits microbial growth through a_w reduction, enhances protein solubilization and regulates proteolysis and lipolysis reactions, what causes an impact on ham texture and flavour (Toldrá and Flores, 1998). Salt is the main source of *Staphylococcus* and *Micrococcus* in dry-cured ham and exerts a selective pressure on the types and counts of microorganisms (Cordero and Zumalacárregui, 2000). The prevalence of *Staphylococcus* over *Micrococcus* in cured meat products is due to its greater resistance to high salt concentration and low redox potential (Lorenzo et al., 2012). *Micrococcaceae* were the predominant microbial group in Parma ham (Hinrichsen and Pedersen, 1995). Also, growth of Gram-positive catalase-positive cocci in Iberian dry-cured hams with the microbial deep spoilage defect was favoured at high salt concentrations (Martín et al., 2006). In the present study, high NaCl concentration hams had the highest counts of aerobic mesophiles, psychrotrophs, and moulds and yeasts, while medium NaCl concentration hams showed the highest counts of lactic acid bacteria. No significant effect of NaCl concentration on *Micrococcaceae* counts was recorded, although the counts tended to be higher in hams of high NaCl concentration and high salt-in-lean ratio.

Intramuscular fat contributes to improve the flavour, texture, mouth-feel, juiciness and lubricity of dry-cured ham. To our knowledge, there is no available information on the effect of intramuscular fat content on the microbiota of Serrano ham. In the present study, higher levels of aerobic mesophiles, psychrotrophs, and moulds and yeasts were recorded in untreated hams of low fat content than in those of medium or high fat content. Higher levels of aerobic mesophiles, *Micrococcaceae*, halophiles and yeasts in fat than in lean of Italian hams were found during the first 4 months at 8 °C, but no differences in microbial counts between lean and fat were recorded at the end of ripening (Giolitti et al., 1971).

In the present study, all microbial groups were significantly ($P < 0.05$) affected by HPP with reductions exceeding 1 log cfu g^{-1} for most of the studied microbial groups. These results are in agreement with those previously reported for microbial counts in slices of vacuum-packaged dry-cured ham treated at 600 MPa for 6 min (Clariana et al., 2011; Garriga et al., 2004). Although a higher sensitivity of psychrotrophs to pressure in comparison with aerobic mesophiles has been reported by some authors (Garriga et al., 2004; Yuste et al., 2000), we did not find that effect. In dry-cured loin HPP-treated at 350 and 400 MPa for 10 min, a significant reduction of aerobic mesophile counts was recorded (Campus et al., 2008). In vacuum-packaged "cecina", HPP at 500 MPa for 5 min lowered *Micrococcaceae*, mould and yeast counts by 2 log cfu g^{-1} and was effective in delaying the growth of spoilage microorganisms (Rubio et al., 2007).

Culture-independent PCR-DGGE method was applied to profile the microbial community of different samples, and the most relevant bands were selected for microbial identification. In spite of the

Table 4
Counts[1] of the main microbial groups in untreated and HPP-treated Serrano ham as influenced by intramuscular fat content.[2]

	Untreated ham				HPP-treated ham			
Microbial group	Low fat	Medium fat	High fat	P^3	Low fat	Medium fat	High fat	P^3
Aerobic mesophiles	3.94 ± 1.38^{a}	2.78 ± 0.78^{ab}	2.40 ± 1.28^{b}	*	1.46 ± 0.60^{a}	1.45 ± 1.05^{a}	1.52 ± 0.45^{a}	ns
Psychrotrophs	4.08 ± 1.48^{a}	2.68 ± 0.92^{b}	2.06 ± 1.17^{b}	**	1.31 ± 0.44^{a}	1.45 ± 1.03^{a}	1.15 ± 0.41^{a}	ns
Lactic acid bacteria	1.49 ± 0.76^{a}	1.40 ± 0.62^{a}	1.82 ± 0.66^{a}	ns	1.00 ± 0.00^{a}	1.06 ± 0.22^{a}	1.30 ± 0.56^{a}	ns
Micrococcaceae	2.61 ± 1.04^{a}	2.01 ± 0.80^{a}	1.63 ± 1.16^{a}	ns	1.12 ± 0.42^{a}	1.42 ± 0.96^{a}	1.00 ± 0.00^{a}	ns
Moulds and yeasts	3.92 ± 1.24^{a}	2.60 ± 0.86^{b}	2.19 ± 1.12^{b}	**	1.07 ± 0.22^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	ns

^{a,b}Means in the same row followed by different superscripts differ ($P < 0.05$).
[1] Counts are expressed in log cfu g^{-1} (mean ± SD of duplicate determinations).
[2] Low intramuscular fat content was <3.56%, medium intramuscular fat content was within the range 3.56–6.99%, and high intramuscular fat content was >6.99%.
[3] Statistical significance in ANOVA: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5
Counts[1] of the main microbial groups in untreated and HPP-treated Serrano ham as influenced by HPP.

Microbial group	Untreated ham	HPP-treated ham	P[2]
Aerobic mesophiles	3.10 ± 1.31[a]	1.47 ± 0.75[b]	***
Psychrotrophs	3.03 ± 1.45[a]	1.32 ± 0.70[b]	***
Lactic acid bacteria	1.55 ± 0.69[a]	1.11 ± 0.33[b]	**
Micrococcaceae	2.35 ± 1.06[a]	1.20 ± 0.65[b]	***
Moulds and yeasts	2.97 ± 1.29[a]	1.02 ± 0.14[b]	***

[a,b]Means in the same row followed by different superscripts differ ($P < 0.05$).
[1] Counts are expressed in log cfu g^{-1} (mean ± SD of duplicate determinations).
[2] Statistical significance in ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 6
Identification of bands obtained from the bacterial community of untreated and HPP-treated Serrano ham after DGGE analysis of the 16S rRNA gene V3 region.

Band[1]	Sample	Closest sequence relative	Identity (%)
1	Untreated/HPP-treated	Mitochondria *Sus scrofa*	93
2	Untreated/HPP-treated	Mitochondria *Sus scrofa*	93
4	Untreated/HPP-treated	*Cellulosimicrobium* sp.	87
6	Untreated	*Bacillus subtilis*	100
7	Untreated	*Bacillus* sp.	98
8	Untreated	Uncultured *Staphylococcus*	97
16	Untreated/HPP-treated	*Staphylococcus equorum*	99
17	HPP-treated	*Staphylococcus equorum*	98
18	Untreated/HPP-treated	*Staphylococcus succinus*	95

[1] Numbers refer to the bands in Fig. 1.

limitations of this technique, the taxonomic identification obtained from the analysis of the ribosomal DNA sequences was considered informative for the aim of this study, i.e. evaluate the effect of the HPP treatment on Serrano ham microbial community. Gram-positive, catalase-positive cocci were identified as the predominant microorganisms along ripening of different types of dry-cured ham (Giolitti et al., 1971; Huerta et al., 1988). In the present study, *S. equorum*, *S. succinus*

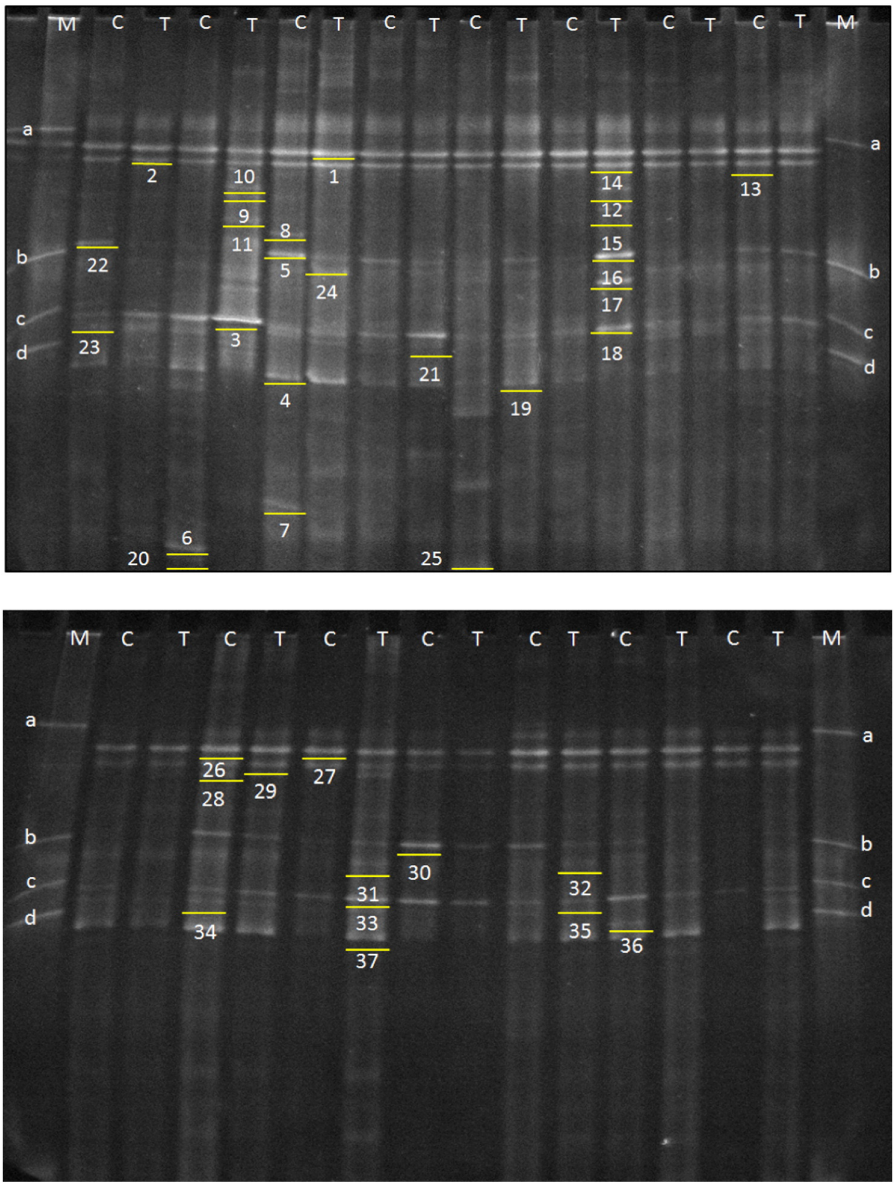


Fig. 1. DGGE profile of the amplified 16S rRNA gene V3 region directly obtained from Serrano ham samples. M = combined amplicons of identified strains used as marker: *Staphylococcus capitis* ssp. *capitis* (a), *Staphylococcus haemolyticus* (b), *Gluconobacter cerinus* (c) and *Bacillus siamensis* (d), C = untreated ham samples and T = HPP-treated ham samples. For the explanation of band numbers see Table 6.

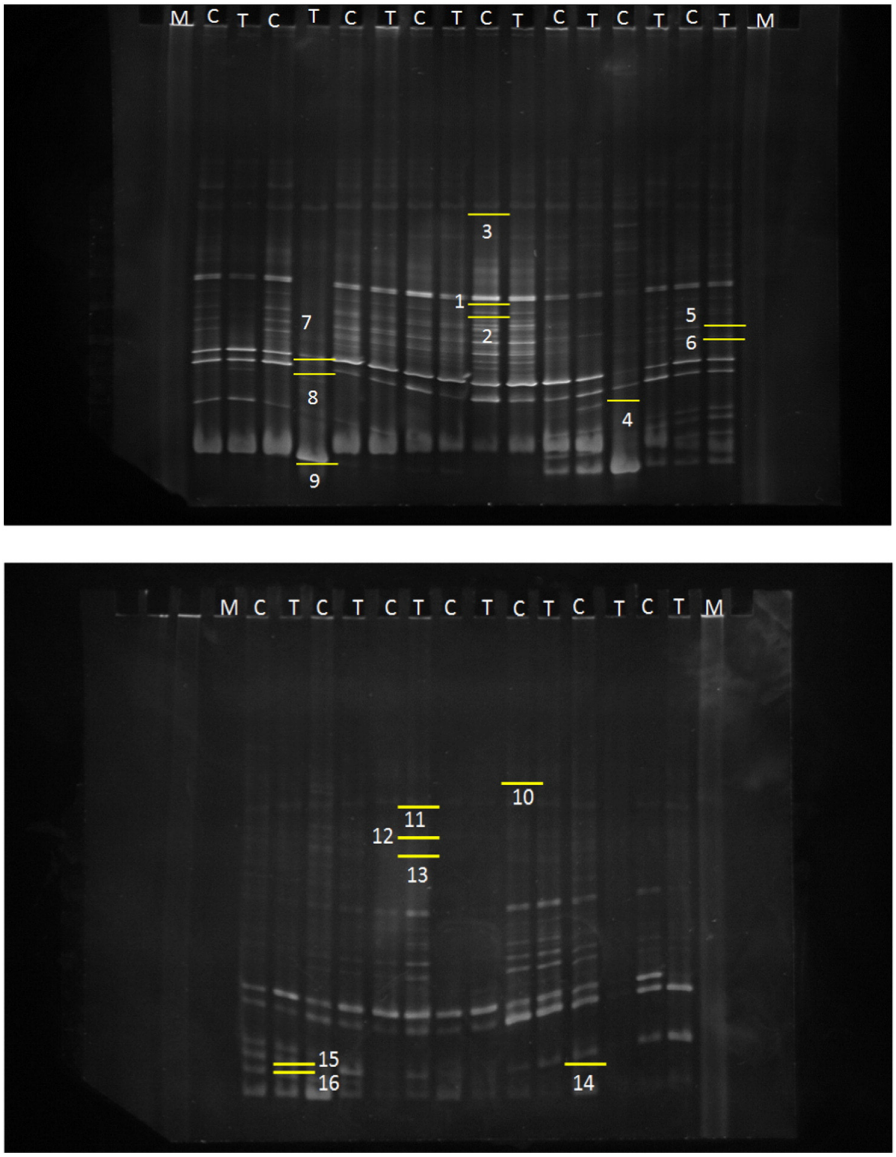


Fig. 2. DGGE profile of the amplified 18S rRNA gene 5′-end region directly obtained from Serrano ham samples. M = marker, C = untreated ham samples and T = HPP-treated ham samples. For the explanation of band numbers see Table 7.

Table 7
Identification of bands obtained from the eukaryotic community of untreated and HPP-treated Serrano ham after DGGE analysis of the 18S rRNA gene 5′-end region.

Band[1]	Sample	Closest sequence relative	Identity (%)
1	Untreated/HPP-treated	*Debaryomyces hansenii*	99
2	Untreated/HPP-treated	*Candida glucosophila*	99
3	Untreated/HPP-treated	Uncultured *Debaryomyces*	99
4	Untreated/HPP-treated	*Penicillium commune*	99
5	Untreated/HPP-treated	*Aspergillus fumigatus*	93
6	Untreated/HPP-treated	*Sclerotinia sclerotiorum*	96
7	Untreated/HPP-treated	*Penicillium commune*	100
10	Untreated/HPP-treated	*Eurotium athecium*	90
11	Untreated/HPP-treated	*Candida glucosophila*	99
12	Untreated/HPP-treated	Uncultured *Debaryomyces*	99
13	Untreated/HPP-treated	*Candida glucosophila*	99
14	Untreated/HPP-treated	*Moniliella mellis*	98

[1] Numbers refer to the bands in Fig. 2.

and *B. subtilis* were the main bacterial species within the microbiota of untreated Serrano ham, but *B. subtilis* bands could not be found in HPP-treated ham samples. This is in agreement with the previous finding that bacilli seem to be more sensitive than cocci to HPP (Hugas et al., 2002). *Staphylococcus* species were prevalent over other *Micrococcaceae* during the processing and ripening of Spanish dry-cured hams (Carrascosa and Cornejo, 1991; Cordero and Zumalacárregui, 2000), because of their resistance to high NaCl concentration and low redox potential (Cordero and Zumalacárregui, 2000). According to the last authors, the same species of *Staphylococcus*, namely *S. equorum*, was isolated from the salt and the final product. Rodríguez et al. (1994) found that the predominant microorganism in Iberian dry-cured ham after 16 months of ripening was *S. xylosus* followed by *S. equorum*. Nevertheless, *S. xylosus* was the prevalent species identified by biochemical methods (87.5%) in industrial Spanish dry-cured ham, while *S. equorum* (73.2%) and *S. vitulinus* (8.9%) were the prevalent species detected by 16S rRNA sequencing (Landeta et al., 2011). The presence of *S. equorum* in ham may have been under-estimated because of its confusion with *S.*

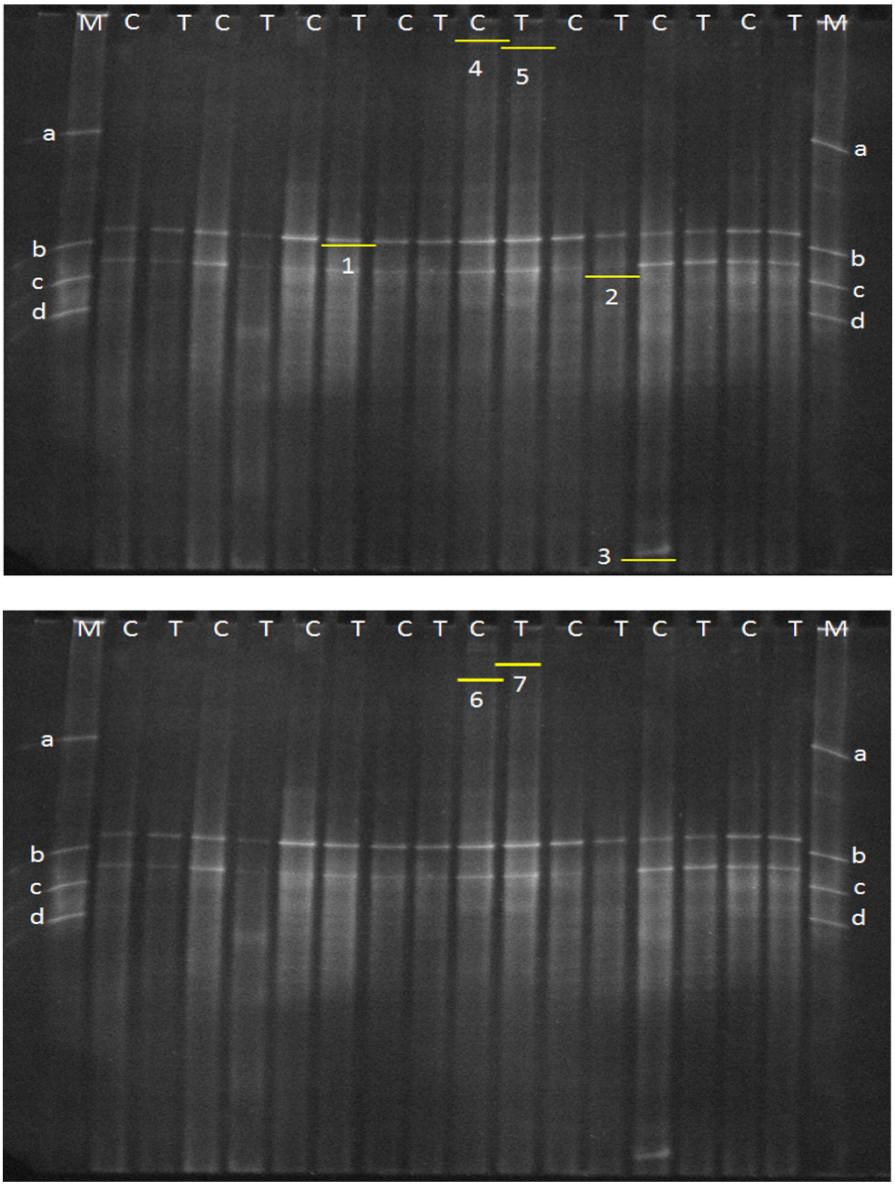


Fig. 3. DGGE profile of the amplified 26S rRNA gene D1 domain directly obtained from Serrano ham samples. M = combined amplicons of identified strains used as marker: *Hanseniaspora uvarum* (a), *Aureobasidium pullulans* (b), *Eremothecium coryli* (c) and *Zygosaccharomyces bisporus* (d), C = untreated ham samples and T = HPP-treated ham samples. For the explanation of band numbers see Table 8.

xylosus when using phenotypical and biochemical identification methods (Blaiotta et al., 2004; Landeta et al., 2013; Mauriello et al., 2004). Studies on the inactivation of the pathogen *S. aureus* in dry-cured ham have shown a decrease of only 0.5 log units after an HPP treatment at 600 MPa for 6 min at 31 °C (Jofré et al., 2009). These results are in agreement with the staphylococcal resistance that we found in HPP-treated Serrano ham samples. No records of the presence of *Bacillus subtilis* or *Cellulosimicrobium* in dry-cured ham have been found in literature. Most studies on *Bacillus* inactivation have been directed towards spore inactivation, and a superior pressure resistance for sporulating microorganisms was observed in meat batters (Moerman et al., 2001). Species of the genus *Cellulosimicrobium* have been described as opportunistic pathogens or were isolated from soil or sea sediments (Brown et al., 2006, Yoon et al., 2007; Hamada et al., 2016).

Some mould species are considered to be beneficial microorganisms in the ripening of dry-cured meat products due to their positive effects on flavour and appearance (Lücke, 1986). However, toxigenic strains have been found on the surface of dry-cured Iberian ham (Núñez et al., 1996a). Fungi from various types of ripened dry-cured hams have been studied in detail (Martín et al., 2006; Rojas et al., 1991; Sutic et al., 1972). In Spanish dry-cured ham the most frequently isolated genera were *Penicillium* and *Aspergillus* (Núñez et al., 1996a). In the present

Table 8
Identification of bands obtained from the eukaryotic community of untreated and HPP-treated Serrano ham after DGGE analysis of the 26S rRNA gene D1 domain.

Band[1]	Sample	Closest sequence relative	Identity (%)
1	Untreated/HPP-treated	*Debaryomyces hansenii*	100
2	Untreated/HPP-treated	*Candida glucosophila*	99
3	Untreated	*Penicillium chrysogenum*	100

[1] Numbers refer to the bands in Fig. 3.

study, *P. commune*, *A. fumigatus*, *S. sclerotiorum*, *E. athecium* and *M. mellis* were detected in untreated and HPP-treated ham samples and *P. chrysogenum* in one untreated sample. After HPP, the mould population, with the only exception of *E. athecium*, seemed to remain unaltered. These results did not agree with the previous finding that vegetative forms of eukaryotic microorganisms were generally more sensitive to pressure than prokaryotic microorganisms (Hoover et al., 1989; Smelt, 1998). *S. sclerotiorum* was previously detected in dry-cured ham (Núñez et al., 1996a). No references of the presence of *S. sclerotiorum* and *M. mellis* in dry-cured ham were found in literature, and their possible contribution to dry-cured ham characteristics is unknown.

Some yeast species contribute to the sensory characteristics of dry-cured ham due to their proteolytic activities (Molina and Toldrá, 1992; Rodríguez et al., 1998) and their role in volatile compound generation (Andrade et al., 2009). In the present study, *D. hansenii* and *C. glucosophila* were found to be members of Serrano ham yeast community. The ability of *D. hansenii* to grow at extremely high NaCl concentrations and low a_w has been reported (Fleet, 1992), a physiological trait which permits its growth and survival in dry-cured ham. In Iberian dry-cured ham, *C. zeylanoides* was reported as the main species at the fresh stage while *D. hansenii* dominated the yeast population after the post salting stage (Núñez et al., 1996b). In Parma dry-cured ham, the predominant species during the whole maturation process were *D. hansenii*, *C. zeylanoides*, *D. maramus* and, to a lesser extent, *C. famata* and *Hyphopichia burtonii* (Simoncini et al., 2007).

In the present study, comparison of control and HPP-treated samples showed slight variations in the Serrano ham microbial community, pointing to a certain baroresistance of the detected species.

5. Conclusions

Chemical composition significantly influenced the levels of aerobic mesophiles, psychrotrophs, lactic acid bacteria, and moulds and yeasts in Serrano dry-cured ham whereas no effect of chemical composition on *Micrococcaceae* was observed. Hams with high NaCl concentration and low intramuscular fat content had the highest counts of aerobic mesophiles, psychrotrophs, and moulds and yeasts. The higher levels of these microorganisms in lean ham could result in a quicker spoilage than in medium or high fat ham. HPP significantly lowered the counts of all studied microbial groups, confirming the efficacy of this treatment against spoilage microorganisms. The microbiota of Serrano ham is mostly composed by the bacterial species *S. equorum*, *S. succinus* and *B. subtilis*, and eukaryotic species such as *P. commune*, *P. chrysogenum*, *A. fumigatus*, *S. sclerotiorum*, *E. athecium*, *M. mellis*, *D. hansenii* and *C. glucosophila*. Changes in Serrano ham microbiota caused by HPP, as determined by DGGE analysis, consisted primarily in the disappearance of *B. subtilis* bands and in weaker bands of *E. athecium*.

In spite of the reductions observed in all microbial groups after the HPP treatment, DGGE does not seem to be sensitive enough to highlight the changes that take place in this microbial community after the HPP treatment. However, DGGE allowed to detect microbial species not previously found in dry-cured ham.

Acknowledgements

The authors wish to thank J. Arnau, P. Gou and E. Fulladosa (IRTA, Monells, Spain) for the selection and processing of hams, the Spanish Ministry of Science and Innovation for supporting the experimental work through project RTA2010-00029-C04-04, and INIA for the Ph. D. grant and the short-term stay of N. Martínez-Onandi.

References

Altschul, S.F., Madden, T.L., Schaeffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Andrade, M.J., Córdoba, J.J., Sánchez, B., Casado, E.M., Rodríguez, M., 2009. Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. Food Chem. 113, 457–463.

AOAC, 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland.

Aymerich, T., Picouet, P.A., Monfort, J.M., 2008. Decontamination technologies for meat products. Meat Sci. 78, 114–129.

Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R., Parente, E., 2004. Diversity and dynamics of communities of coagulase-negative *Staphylococci* in traditional fermented sausages. J. Appl. Microbiol. 97, 271–284.

Blesa, E., Aliño, M., Barat, J.M., Grau, R., Toldrá, F., Pagán, M.J., 2008. Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. Meat Sci. 78, 135–142.

Brown, J.M., Steigerwalt, A.G., Morey, R.E., Daneshvar, M.I., Romero, L.J., McNeil, M.M., 2006. Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp nov and emended description of the genus *Cellulosimicrobium*. Int. J. Syst. Evol. Microbiol. 56, 801–804.

Campos, G., Robles, L., Alonso, R., Nuñez, M., Picon, A., 2011. Microbial dynamics during the ripening of a mixed cow and goat milk cheese manufactured using frozen goat milk curd. J. Dairy Sci. 94, 4766–4776.

Campus, M., Flores, M., Martinez, A., Toldrá, F., 2008. Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. Meat Sci. 80, 1174–1181.

Carrascosa, A.V., Cornejo, I., 1991. Characterization of *Micrococcaceae* strains selected as potential starter cultures to Spanish dry-cured ham processes. II: slow process. Fleischwirtschaft 71, 1187–1188.

Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, A., García-Regueiro, J.A., 2011. Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects along the storage period. Innovative Food Sci. Emerg. Technol. 12, 456–465.

Cocolin, L., Bisson, L.F., Mills, D.A., 2000. Direct profiling of the dynamics in wine fermentations. FEMS Microbiol. Lett. 189, 81–87.

Cordero, M.R., Zumalacárregui, J.M., 2000. Characterization of *Micrococcaceae* isolated from salt used for Spanish dry-cured ham. Lett. Appl. Microbiol. 31, 303–306.

Córdoba, J.J., Antequera, T., García, C., Ventanas, J., López Bote, C., Asensio, M.A., 1994. Evolution of free amino acids and amines during ripening of Iberian cured ham. J. Agric. Food Chem. 42, 2296–2301.

Cornejo, I., Carrascosa, A.V., Marin, M.E., Martin, P.J., 1992. Dry-cured ham. Considerations about the origin of microorganisms that grow on the deep muscular tissues of dry-cured Spanish hams during processing. Fleischwirtschaft 72, 1422–1425.

Coutron-Gambotti, C., Gandemer, G., Rousset, S., Maestrini, O., Casabianca, F., 1999. Reducing salt content of dry-cured ham: effect on lipid composition and sensory attributes. Food Chem. 64, 13–19.

Diez, A.M., Urso, R., Rantsiou, K., Jaime, I., Rovira, J., Cocolin, L., 2008. Spoilage of blood sausages *morcilla de Burgos* treated with high hydrostatic pressure. Int. J. Food Microbiol. 123, 246–253.

Duong, L.M., Jeewon, R., Lumyong, S., Hyde, K.D., 2006. DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. Fungal Divers. 23, 121–138.

Fleet, G., 1992. Spoilage yeasts. Crit. Rev. Biotechnol. 12, 1–44.

Folch, J., Lees, M., Sloane-Stanley, G.H.S., 1957. A simple method from the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Garriga, M., Grèbol, N., Aymerich, M.T., Monfort, J.M., Hugas, M., 2004. Microbial inactivation after high pressure processing at 600 MPa in commercial meat products over its shelf life. Innovative Food Sci. Emerg. Technol. 5, 451–457.

Giolitti, G., Cantoni, C., Bianchi, M., Renon, P., Beretta, G., 1971. Microbiology and chemical changes in raw hams of Italian type. J. Appl. Microbiol. 34, 51–61.

Hamada, M., Shibata, C., Tamura, T., Nurkanto, A., Ratnakomala, S., Lisdiyanti, P., Suzuki, K., 2016. *Cellulosimicrobium marinum* sp nov., an actinobacterium isolated from sea sediment. Arch. Microbiol. 198, 439–444.

Han, Y.Q., Jiang, Y., Xu, X.L., Sun, X.S., Xu, B.C., Zhou, G.H., 2011. Effect of high pressure treatment on microbial populations of sliced vacuum-packed cooked ham. Meat Sci. 88, 682–688.

Hinrichsen, L.L., Pedersen, S.B., 1995. Relationship among flavor, volatile compounds, chemical-changes, and microflora in Italian-type dry-cured ham during processing. J. Agric. Food Chem. 43, 2932–2940.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., Knorr, D., 1989. Biological effects on high hydrostatic pressure on food microorganisms. Food Technol. 43, 99–107.

Huerta, T., Hernandez, J., Guamis, B., Hernandez, E., 1988. Microbiological and physico-chemical aspects in dry-salted Spanish ham. Zentralbl. Mikrobiol. 143, 475–482.

Hugas, M., Garriga, M., Monfort, J.M., 2002. New mild technologies in meat processing: high pressure as a model technology. Meat Sci. 62, 359–371.

Jofré, A., Aymerich, T., Grèbol, N., Garriga, M., 2009. Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products. LWT Food Sci. Technol. 42, 924–928.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J., 2012. Introducing EzTaxon: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int. J. Syst. Evol. Microbiol. 62, 716–721.

Landeta, G., Reverón, I., Carrascosa, A.V., de las Rivas, B., Muñoz, R., 2011. Use of *recA* gene sequence analysis for the identification of *Staphylococcus equorum* strains predominant on dry-cured hams. Food Microbiol. 28, 1205–1210.

Landeta, G., Curiel, J., Carrascosa, A., Muñoz, R., de las Rivas, B., 2013. Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products. Meat Sci. 93, 387–396.

Langlois, B.E., Kemp, J.D., 1974. Microflora of fresh and dry-cured hams as affected by fresh ham storage. J. Anim. Sci. 38, 525–531.
Lorenzo, J.M., Fontán, M.C.G., Gómez, M., Fonseca, S., Franco, I., Carballo, J., 2012. Study of the *Micrococcaceae* and *Staphylococcaceae* throughout the manufacture of dry-cured Lacón (a Spanish traditional meat product) made without or with additives. J. Food Res. 1, 200–211.
Lücke, F.K., 1986. Microbiological processes in the manufacture of dry sausage and raw ham. Fleischwirtschaft 66, 1505–1509.
Martín, A., Córdoba, J.J., Rodríguez, M.M., Nuñez, F., Asensio, M.A., 2001. Evaluation of microbial proteolysis in meat products by capillary electrophoresis. J. Appl. Microbiol. 90, 163–171.
Martín, A., Córdoba, J.J., Aranda, E., Córdoba, M.G., Asensio, M.A., 2006. Contribution of a selected fungal population to the volatile compounds on dry-cured ham. Int. J. Food Microbiol. 110, 8–18.
Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., Picon, A., 2016. Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. Meat Sci. 111, 130–138.
Mauriello, G., Casaburi, A., Blaiotta, G., Villani, F., 2004. Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. Meat Sci. 67, 149–158.
May, L.A., Smiley, B., Schmidt, M.G., 2001. Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Can. J. Microbiol. 47, 829–841.
Moerman, F., Mertens, B., Demey, L., Huyghebaert, A., 2001. Reduction of *Bacillus subtilis*, *Bacillus stearothermophilus* and *Streptococcus faecalis* in meat batters by temperature-high hydrostatic pressure pasteurization. Meat Sci. 59, 115–125.
Molina, I., Toldrá, F., 1992. Detection of proteolytic activity in microorganisms isolated from dry-cured ham. J. Food Sci. 57, 1308–1310.
Monte, E., Villanueva, J.R., Domínguez, A., 1986. Fungal profiles of Spanish country-cured hams. Int. J. Food Microbiol. 3, 355–359.
Morales, P., Calzada, J., Nuñez, M., 2006. Effect of high pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. J. Food Prot. 69, 2539–2543.
Ng, W.F., Langlois, B.E., Moody, W.G., 1997. Fate of selected pathogens in vacuum-packaged dry-cured (country-style) ham slices stored at 2 and 25 °C. J. Food Prot. 60, 1541–1547.
Núñez, F., Rodríguez, M.M., Bermúdez, E., Córdoba, J.J., Asensio, M.A., 1996a. Composition and toxigenic potential of mould population on dry-cured Iberian ham. Int. J. Food Microbiol. 32, 185–197.
Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E., Asensio, M.A., 1996b. Yeast population during ripening of dry-cured Iberian ham. Int. J. Food Microbiol. 29, 271–280.
Patterson, M.F., 2005. Microbiology of pressure-treated foods. J. Appl. Microbiol. 98, 1400–1409.
Rodríguez, M., Núñez, F., Córdoba, J.J., Sanabria, C., Bermúdez, E., Asensio, M.A., 1994. Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. Int. J. Food Microbiol. 85, 905–912.
Rodríguez, M., Núñez, F., Córdoba, J.J., Sanabria, C., Bermúdez, E., Asensio, M.A., 1998. Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. J. Appl. Microbiol. 85, 905–912.
Rojas, F.J., Jodral, M., Gosalvez, F., Pozo, R., 1991. Mycoflora and toxigenic *Aspergillus flavus* in Spanish dry-cured ham. Int. J. Food Microbiol. 13, 249–255.
Rubio, B., Martínez, B., García-Cachan, M.D., Rovira, J., Jaime, I., 2007. Effect of high pressure preservation on the quality of dry cured beef "Cecina de Leon". Innovative Food Sci. Emerg. Technol. 8, 102–110.
Simoncini, N., Rotelli, D., Virgili, R., Quintavalla, S., 2007. Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. Food Microbiol. 24, 577–584.
Smelt, J.P.P.M., 1998. Recent advances in the microbiology of high pressure processing. Trends Food Sci. Technol. 9, 152–158.
Sutic, M., Ayres, J.C., Koehler, P.E., 1972. Identification and aflatoxin production of moulds isolated from country cured hams. Appl. Microbiol. 23, 656–658.
Toldrá, F., Flores, M., 1998. The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. Crit. Rev. Food Sci. Nutr. 38, 331–352.
Walter, J., Tannock, G.W., Tilsala-Timisjarvi, A., 2000. Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl. Environ. Microbiol. 66, 297–303.
White, T.J., Bruns, T.D., Lee, S., Taylor, J., 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322.
Yoon, J.H., Kang, S.J., Schumann, P., Oh, T.K., 2007. *Cellulosimicrobium terreum* sp nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 57, 2493–2497.
Yuste, J., Pla, R., Capellas, M., Ponce, E., Mor-Mur, M., 2000. High-pressure processing applied to cooked sausages: bacterial populations during chilled storage. J. Food Prot. 63, 1093–1099.

5. Influence of physicochemical characteristics and high pressure processing on the volatile fraction of Iberian dry-cured ham

Artículo publicado en *Meat Science* 131 (2017) 40-47

Meat Science 131 (2017) 40–47

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Influence of physicochemical characteristics and high pressure processing on the volatile fraction of Iberian dry-cured ham

Nerea Martínez-Onandi, Ana Rivas-Cañedo, Marta Ávila, Sonia Garde, Manuel Nuñez*, Antonia Picon

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, Madrid 28040, Spain

ARTICLE INFO

Keywords:
Iberian ham
Volatile compound
Intramuscular fat
Salt
Water activity
High pressure processing

ABSTRACT

The volatile fraction of 30 Iberian dry-cured hams of different physicochemical characteristics and the effect of high pressure processing (HPP) at 600 MPa on volatile compounds were investigated. According to the analysis of variance carried out on the levels of 122 volatile compounds, intramuscular fat content influenced the levels of 8 benzene compounds, 5 carboxylic acids, 2 ketones, 2 furanones, 1 alcohol, 1 aldehyde and 1 sulfur compound, salt concentration influenced the levels of 1 aldehyde and 1 ketone, salt-in-lean ratio had no effect on volatile compounds, and water activity influenced the levels of 3 sulfur compounds, 1 alcohol and 1 aldehyde. HPP-treated samples of Iberian ham had higher levels of 4 compounds and lower levels of 31 compounds than untreated samples. A higher influence of HPP treatment on volatile compounds than physicochemical characteristics was observed for Iberian ham. Therefore, HPP treatment conditions should be optimized in order to diminish its possible effect on Iberian ham odor and aroma characteristics.

1. Introduction

Iberian dry-cured ham is manufactured in Spain from the hind legs of Iberian breed pigs. The traditional process consists in the addition of curing salts, which diffuse into the ham during the post-salting stage, followed by a long ripening-dehydration stage lasting up to 48 months during which its flavor and aroma develop.

The unique distinctive aroma of Iberian ham is partly derived from the rich lipid fraction characterizing the meat of this autochthonous animal breed (Andrés, Cava, Martin, Ventanas, & Ruiz, 2005). In dry-cured ham, free fatty acids are generated from the lipids in muscle and adipose tissue while small peptides and free amino acids derive from muscle proteins (Toldrá & Flores, 1998; Zhang, Jin, Wang, & Zhang, 2011). Part of these intermediate compounds is transformed into volatile compounds through lipid oxidation, Maillard reactions, Strecker degradation and other reactions (García et al., 1991; López et al., 1992; Zhang, Zhen, Zhang, Zeng, & Zhou, 2009). Chemical composition of meat, added curing salts and processing conditions affect the formation and stability of volatile compounds in dry-cured ham.

Current consumer trends lead towards the production of low-salt low-fat dry-cured ham. However, changes in the chemical composition of ham could impair its microbial stability, affect its shelf-life and influence its volatile fraction (Armenteros, Toldrá, Aristoy, Ventanas, & Estévez, 2012; Blesa et al., 2008). Proteolysis, lipolysis and lipid oxidation phenomena might be influenced by variations in physicochemical characteristics such as salt concentration, water activity (a_w) and fat content and the generation of aroma compounds might thus be altered. Salt concentration influenced the formation of some volatile compounds in Iberian ham (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004a; Andrés, Cava, Ventanas, Muriel, & Ruiz, 2007). In dry-cured turkey ham, salt concentration correlated positively with total aldehydes content and negatively with alcohols, ketones and alkanes contents (Wang, Jin, Zhang, Ahn, & Zhang, 2012). Regarding the influence of fat content on the formation of volatile compounds, different volatile profiles were found between muscles and subcutaneous fat of dry-cured hams (Sánchez-Peña, Luna, García-González, & Aparicio, 2005). A higher abundance of volatile compounds was found in Iberian dry-cured loins of high intramuscular fat content than in those of low intramuscular fat content (Ventanas, Estevez, Andrés, & Ruiz, 2008). Also, the levels of 16 out of 39 volatile compounds differed between Iberian hams from pigs with different fatty acid composition of the intramuscular fat (Carrapiso et al., 2015).

Ready-to-eat sliced meat products have gained worldwide consumer acceptance. However, pathogens and spoilage microorganisms may reach the product during the deboning, slicing and packaging of ready-to eat dry-cured ham, affecting its safety and shelf-life. For this reason, high pressure processing (HPP) is widely used at the meat industry to eliminate undesirable contaminating microorganisms (Clariana et al.,

* Corresponding author.
E-mail address: nunez@inia.es (M. Nuñez).

http://dx.doi.org/10.1016/j.meatsci.2017.04.233
Received 23 January 2017; Received in revised form 24 April 2017; Accepted 25 April 2017
Available online 25 April 2017

2011; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004).

HPP treatment is also known to affect the volatile compounds of Serrano dry-cured ham (Rivas-Cañedo, Fernández-García, & Nuñez, 2009) and the lipid oxidation phenomena of Iberian dry-cured ham (Andrés, Adamsen, Møller, Ruiz, & Skibsted, 2006; Andrés, Møller, Adamsen, & Skibsted, 2004b; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010).

The effect of HPP on the volatile fraction of Serrano hams of different chemical composition was recently studied (Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016). However, information on the effect of HPP treatment on the volatile compounds of Iberian ham is scarce. To our knowledge, only the effect of HPP treatment on the volatile aldehydes of Iberian ham has been reported (Fuentes et al., 2010). Information on the influence of the physicochemical characteristics of Iberian ham on its volatile fraction is also limited. Iberian ham has higher intramuscular fat content, lower salt concentration and salt-in-lean ratio, and higher a_w than Serrano ham, compositional characteristics which might influence the formation of volatile compounds during manufacture and ripening as well as the effect of HPP treatment. The objective of the present study was to investigate the influence of those physicochemical characteristics and HPP treatment on the volatile fraction of Iberian dry-cured ham.

2. Material and methods

2.1. Selection of Iberian hams

Thirty Iberian hams from 50% Iberian × Duroc animals, manufactured and ripened at a processing plant in Extremadura (Spain), were selected at the Institute of Food and Agricultural Research (IRTA, Monells, Spain) on the basis of their subcutaneous fat thickness and their salt content estimated by means of computed tomography (Santos-Garcés, Gou, García-Gil, Arnau, & Fulladosa, 2010) as previously described (Martínez-Onandi et al., 2016).

2.2. Sampling and high pressure processing

Two slices (150–200 g in weight) obtained from the cushion of each ham (mainly composed of the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* muscles) were individually vacuum-packaged in polyamide + polyethylene bags (Mobepack, Salamanca, Spain). One of the slices was HPP-treated at 600 MPa for 6 min at 21 °C as previously described (Martínez-Onandi et al., 2016). The other slice served as untreated control. Both slices were held at 4 °C for 3 days and afterwards kept at − 35 °C until analysis, for not > 30 days.

2.3. Physicochemical determinations

Prior to analysis, a representative 100 g portion of each ham slice was minced by means of a mechanical grinder (IKA Labortechnik, Staufen, Germany). Intramuscular fat content was determined by the Folch method (Folch, Lees, & Slone Stanley, 1957), chloride content by the Volhard method (AOAC, 2000) and a_w by means of an AquaLab Series 3 equipment (Decagon Devices Inc., Pullman, WA, USA). All analyses were performed in triplicate.

2.4. Analysis of volatile compounds

A representative 15 g portion of each ham slice was homogenized in a mechanical grinder (IKA Labortechnik) with 15 g of anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) and 30 μL of an aqueous solution of 534 mg/L cyclohexanone (Sigma-Aldrich, Alcobendas, Spain) as internal standard. Volatile compounds were extracted by solid-phase microextraction (SPME), analyzed by gas chromatography–mass spectrometry (GC–MS) and identified as previously described (Martínez-Onandi et al., 2016). Semi-quantitative determination of volatile compounds and expression of results was as indicated by those authors. Each ham was analyzed in triplicate.

2.5. Statistical analysis

Experimental data were analyzed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA). Three groups of hams of low, medium and high values for each of the physicochemical characteristics were differentiated by using the mean ± 0.5 standard deviations (SD) criterion (Martínez-Onandi et al., 2016). Analysis of variance was carried out with each of the physicochemical characteristics as main effect and ham as random effect or with HPP treatment as main effect and ham as fixed effect. Tukey's test with the significance assigned at $P < 0.05$ was used for the comparison of means. Principal component analysis (PCA), with Varimax rotation, was carried out on total levels of chemical groups of volatile compounds, levels of individual volatile compounds, physicochemical characteristics and HPP treatment.

3. Results and discussion

3.1. Iberian ham volatile compounds

Volatile compounds detected in the present study included 10 carboxylic acids, 18 alcohols, 11 aldehydes, 18 ketones, 10 esters, 13 alkanes, 23 benzene compounds, 5 sulfur compounds, 4 furanes, 5 furanones, 3 pyrazines and 2 miscellaneous compounds (Table 1). These 122 volatile compounds were found in all control and HPP-treated samples.

The volatile fraction of Iberian ham has been the subject of previous studies (García et al., 1991; Ruiz, Cava, Ventanas, & Jensen, 1998). Factors such as animal genotype (Ramírez & Cava, 2007), animal diet (López et al., 1992), ham ripening time (Ruiz, Ventanas, Cava, Andrés, & García, 1999), muscle (Sánchez-Peña et al., 2005), and anatomical location (Narváez-Rivas, Gallardo, Ríos, & León-Camacho, 2010; Timón, Ventanas, Carrapiso, Jurado, & García, 2001) influenced Iberian ham volatile fraction. The number of volatile compounds detected in the above cited studies on Iberian ham ranged from 55 to 109, but the total number of volatile compounds found in the literature on Iberian ham rises to 411 (Narváez-Rivas, Gallardo, & León-Camacho, 2012).

Carboxylic acids reported in previous works on Iberian ham ranged from 1 (García et al., 1991) to 6 (Ramírez & Cava, 2007) in number, alcohols from 7 (Ruíz et al., 1999) to 14 (Sabio, Vidal-Aragón, Bernalte, & Gata, 1998), aldehydes from 12 (Ramírez & Cava, 2007) to 19 (Ruiz et al., 1998; Timón et al., 2001), ketones from 3 (López et al., 1992) to 15 (Sabio et al., 1998), esters from 2 (Ramírez & Cava, 2007) to 10 (Sabio et al., 1998), sulfur compounds from 1 (García et al., 1991) to 8 (Sabio et al., 1998), and nitrogen compounds from 2 (Ruiz, Ventanas, Cava, Andrés, & García, 1999) to 5 (Ruiz et al., 1998). The main reason for those differences in the number of volatile compounds would probably be the analytical procedure used, although ham characteristics due to feeding of animals and manufacturing and ripening conditions might also be involved.

In the present study, compounds presumably derived from lipid oxidation (linear carboxylic acids, linear alcohols, linear aldehydes, linear ketones, and hydrocarbons) accounted for 75.0% of the volatile fraction of untreated Iberian ham, compounds derived from Maillard reactions (branched-chain carboxylic acids, branched-chain alcohols, branched-chain aldehydes, branched-chain ketones, nitrogen compounds and sulfur compounds) for 18.1%, and compounds of microbial or unknown origin (acetic acid and esters) for 6.9% (data not shown). In a previous work on Iberian ham the results obtained were similar, with 81.6% of its volatile fraction considered to derive from lipid oxidation, 12.7% from Maillard reactions and 5.7% from microbial or unknown origin (Ramírez & Cava, 2007).

Not all the volatile compounds present in Iberian ham are equally

Table 1
Volatile compounds identified in untreated and HPP-treated Iberian ham.

Compound	LRI[a]	QI[b]	ID[c]
Acids			
Acetic acid	1469	43, 45, 60	ST, MS
Propanoic acid	1549	45, 57, 74	ST, MS
Butanoic acid	1641	60, 73, 88	ST, MS
Pentanoic acid	1682	43, 60, 87	ST, MS
Hexanoic acid	1855	41, 60, 87	ST, MS
Heptanoic acid	1968	43, 60, 73	ST, MS
Octanoic acid	2063	43, 73, 101, 115	ST, MS
2-Methyl propanoic acid	1578	43, 73, 88	ST, MS
2-Methyl butanoic acid	1679	57, 74	ST, MS
3-Methyl butanoic acid	1681	43, 60	ST, MS
Alcohols			
Ethanol	941	45, 46, 43, 41	ST, MS
1-Propanol	1059	59, 42, 60	ST, MS
1-Butanol	1165	56, 41, 43, 42	ST, MS
1-Pentanol	1265	42, 55, 41, 70	ST, MS
1-Hexanol	1364	56, 55, 69, 41	ST, MS
1-Heptanol	1479	70, 56, 55, 69	ST, MS
1-Octanol	1582	56, 55, 69, 70	ST, MS
2-Propanol	934	45, 43, 41, 59	ST, MS
2-Pentanol	1141	45, 73	ST, MS
2-Hexanol	1236	45, 43, 69, 57	ST, MS
2-Heptanol	1326	45, 70, 83, 98	ST, MS
2-Methyl-1-propanol	1112	42, 43, 41, 74	ST, MS
3-Methyl-1-butanol	1225	55, 70, 42, 43	ST, MS
1-Penten-3-ol	1177	57, 41, 43, 88	ST, MS
1-Octen-3-ol	1471	57, 72, 55	ST, MS
2-Methyl-3-buten-2-ol	1060	53, 59, 65, 71	ST, MS
2-Butoxyethanol	1431	57, 75, 87, 100, 108	ST, MS
1-Methoxy-2-propanol	1148	45, 59, 75, 90	ST, MS
Aldehydes			
Ethanal	704	41, 42, 43, 44	ST, MS
Pentanal	982	41, 4457, 58	ST, MS
Hexanal	1097	56, 57, 72	ST, MS
Heptanal	1199	70, 44, 43, 55	ST, MS
Octanal	1299	56, 57, 84	ST, MS
Nonanal	1413	57, 98, 70, 82	ST, MS
2-Methylpropanal	816	41, 43, 72	ST, MS
2-Methylbutanal	917	57, 41, 58, 39	ST, MS
3-Methylbutanal	921	44, 58, 41, 43	ST, MS
2-Methyl-2-butenal	1101	83, 82	ST, MS
3-(Methylthio)-propanal	1479	47, 48, 76, 104	ST, MS
Ketones			
2-Propanone	819	58, 43, 42, 39	ST, MS
2-Butanone	907	43, 72, 57	ST, MS
2-Pentanone	985	43, 41, 86, 57	ST, MS
2-Hexanone	1089	58, 85, 100	ST, MS
2-Heptanone	1198	43, 58, 71, 85	ST, MS
2-Octanone	1300	43, 58, 71, 59	ST, MS
2-Nonanone	1402	58, 71	ST, MS
3-Heptanone	1164	57, 85, 114	ST, MS
3-Octanone	1262	72, 99, 128	ST, MS
2,3-Pentanedione	1069	43, 57, 100	ST, MS
2,3-Octanedione	1326	43, 71, 99	MS
1-(2-Furanyl)-ethanone	1060	67, 95, 110	MS
1-Hydroxy-2-propanone	1314	43, 74	ST, MS
1-Hydroxy-2-butanone	1392	56, 57, 88	ST, MS
3-Hydroxy-2-butanone	1303	43, 45, 73, 88	ST, MS
3-Ethylcyclopentanone	1348	70, 83, 97, 112	MS
4-Hydroxy-4-methyl-2-pentanone	1379	43, 58, 58	ST, MS
6-Methyl-2-heptanone	1245	58, 110, 113	ST, MS
Esters			
Ethyl acetate	894	43, 45, 61, 70	ST, MS
Ethyl propanoate	957	57, 74, 75, 102	ST, MS
Ethyl butanoate	1052	43, 60, 71, 88, 100	ST, MS
Ethyl 2-methylbutanoate	1070	57, 74, 85, 102, 115	ST, MS
Ethyl 3-methylbutanoate	1084	60, 70, 88, 115, 130	ST, MS
Ethyl hexanoate	1246	55, 60, 70, 88, 99, 115	ST, MS
Ethyl heptanoate	1338	60, 70, 88	ST, MS
Ethyl octanoate	1453	88, 127	ST, MS
Ethyl decanoate	1661	88, 101, 157	ST, MS
Ethyl 2-hydroxypropanoate	1352	45, 75	ST, MS
Alkanes			
Pentane	500	41, 42, 57, 72	ST, MS
Hexane	600	57, 41, 56, 42	ST, MS
Heptane	700	43, 57, 71, 41	ST, MS
Octane	800	43, 57, 85, 71	ST, MS
Nonane	900	41, 56, 57, 85	ST, MS
Decane	1000	43, 57, 71, 85, 99	ST, MS
Hexadecane	1581	85	ST, MS
Cyclohexane	724	56, 84	ST, MS
1-Heptene	735	41, 55, 56, 70	ST, MS
2-Octene	851	41, 55, 56, 83	ST, MS
2,2,4,6,6-Pentamethylheptane	941	57, 56, 71, 85	ST, MS
Branched chain alkane I	1005	71, 85, 111, 127	MS
Branched chain alkane II	1032	57, 71, 113	MS
Benzene compounds			
Benzene	939	51, 52, 77, 78	ST, MS
Methylbenzene	1055	91, 92, 65, 93	ST, MS
1,2,3-Trimethylbenzene	1293	105, 120	ST, MS
Ethylbenzene	1131	91, 106, 65, 51	ST, MS
1-Methylethylbenzene	1183	79, 105, 120	ST, MS
o-Ethyltoluene	1231	105, 120	ST, MS
m-Ethyltoluene	1233	77, 91, 105, 120	ST, MS
Styrene	1272	104, 103, 78, 77	ST, MS
Benzaldehyde	1556	77, 105, 106, 51	ST, MS
Benzonitrile	1644	50, 76, 103	ST, MS
Phenol	2037	66, 94	ST, MS
4-Methyl-phenol	2093	77, 90, 107	ST, MS
2, 4-Dimethyl-phenol	1254	107, 121, 122	ST, MS
4-Ethyl-phenol	1226	77, 107, 122	ST, MS
4-Pentyl-phenol	1497	107, 164	ST, MS
o-Xylene	1199	91, 106, 105, 77	ST, MS
m-Xylene	1157	91, 106, 105, 77	ST, MS
p-Xylene	1150	91, 106	ST, MS
Phenyl-methanol	1923	108, 109	ST, MS
2-Phenyl-ethanol	1970	65, 91, 92, 122	ST, MS
Phenyl-ethanal	1698	43, 60	ST, MS
1-Phenyl-propane	1218	91, 92, 120	ST, MS
Naphthalene	1800	51, 64, 128	ST, MS
Sulfur compounds			
Methanethiol	678	47, 48, 45, 46	ST, MS
Carbon disulfide	729	76, 78, 77, 64	ST, MS
Dimethyl sulfide	747	35, 47, 61, 62	ST, MS
Dimethyl disulfide	1086	94, 79, 46	ST, MS
Dimethyl trisulfide	1402	126, 45, 47, 79	ST, MS
Furanes			
2-Methylfuran	871	51, 81, 82	ST, MS
2-Ethylfuran	952	53, 81, 96, 51, 82	ST, MS
2-Butylfuran	1141	53, 81, 124	ST, MS
2-Pentylfuran	1240	81, 82, 95, 53, 138	ST, MS
Furanones			
Dihydro-2(3H)-furanone	1652	42, 56, 86	ST, MS
5-Methyldihydro-2(3H)-furanone	1673	56, 86	ST, MS
5-Ethyldihydro-2(3H)-furanone	1756	56, 70, 85	ST, MS
5-Butyldihydro-2(3H)-furanone	1865	56, 85	MS
5-Pentyldihydro-2(3H)-furanone	1988	85, 124	MS
Pyrazines			
Methylpyrazine	1285	40, 53, 67, 94	ST, MS
2,6-Dimethylpyrazine	1346	53, 67, 81, 93	ST, MS
2,3,5-Trimethylpyrazine	1437	122, 42, 81, 39	ST, MS
Miscellaneous compounds			
Pyrrole	1534	38, 66, 67	ST, MS
p-Nitrophenyl hexanoate	1696	43, 55, 71, 99	ST, MS

[a] Linear retention indexes, calculated in relation to the retention time of n-alkane (C5–C20) series.
[b] QI: Ions used for quantification.
[c] ID: Peak identification: ST, comparison of spectra and retention time with commercial standards; MS, tentatively identified by spectra comparison using the Wiley Library.

Table 2
Levels[1] of the 20 volatile compounds significantly influenced by intramuscular fat content in untreated or HPP-treated Iberian ham.

Compound	Untreated ham				HPP-treated ham			
	Low fat (n = 9) < 10.18%	Medium fat (n = 10) 10.18–13.75%	High fat (n = 11) > 13.75%	P^2	Low fat (n = 9) < 10.18%	Medium fat (n = 10) 10.18–13.75%	High fat (n = 11) > 13.75%	P^2
Acetic acid	618.90 ± 32.44^a	589.99 ± 20.88^a	677.49 ± 38.41^a	ns	573.27 ± 24.60^b	460.35 ± 26.56^a	644.22 ± 30.40^b	***
Propanoic acid	21.11 ± 1.00^a	20.76 ± 0.75^a	22.27 ± 1.54^a	ns	19.63 ± 0.78^{ab}	16.39 ± 1.37^a	20.88 ± 1.12^b	*
Butanoic acid	251.17 ± 17.95^a	229.78 ± 16.83^a	210.88 ± 15.07^a	ns	230.02 ± 13.59^b	174.91 ± 12.53^a	204.46 ± 16.36^{ab}	*
Hexanoic acid	372.80 ± 25.39^b	365.06 ± 19.68^b	278.20 ± 16.90^a	**	351.62 ± 34.79^a	283.25 ± 24.34^a	286.15 ± 27.08^a	ns
Octanoic acid	19.96 ± 1.11^a	17.22 ± 1.32^a	17.77 ± 1.34^a	ns	19.10 ± 0.75^b	13.31 ± 1.36^a	16.46 ± 1.31^{ab}	**
1-Pentanol	170.52 ± 9.41^b	178.80 ± 5.70^b	140.23 ± 9.07^a	**	175.08 ± 15.10^a	186.08 ± 15.86^a	146.88 ± 7.57^a	ns
Octanal	13.30 ± 1.39^a	12.71 ± 1.32^a	11.62 ± 1.18^a	ns	11.22 ± 0.96^a	17.81 ± 1.83^b	12.51 ± 1.06^a	**
1-(2-Furanyl)-ethanone	3.62 ± 0.22^b	3.69 ± 0.19^b	2.90 ± 0.22^a	*	3.73 ± 0.25^b	3.68 ± 0.14^b	3.01 ± 0.17^a	*
3-Ethylcyclopentanone	11.59 ± 0.60^b	11.07 ± 0.32^{ab}	9.98 ± 0.38^a	*	12.09 ± 0.83^a	11.28 ± 0.67^a	10.22 ± 0.39^a	ns
Benzene	8.37 ± 0.54^b	8.23 ± 0.59^{ab}	6.59 ± 0.32^a	*	9.06 ± 0.61^a	8.62 ± 0.78^a	7.21 ± 0.50^a	ns
Benzaldehyde	115.27 ± 6.86^b	107.74 ± 7.14^{ab}	92.79 ± 4.04^a	*	116.43 ± 8.38^a	101.26 ± 5.89^a	94.90 ± 3.74^a	ns
Ethyl-phenol	36.06 ± 1.85^b	38.76 ± 2.44^b	27.93 ± 1.96^a	**	37.11 ± 2.14^b	38.02 ± 2.53^b	29.36 ± 1.73^a	*
m-Ethyltoluene	3.98 ± 0.37^a	4.23 ± 0.57^{ab}	6.31 ± 0.77^b	*	4.03 ± 0.44^a	4.24 ± 0.64^{ab}	6.35 ± 0.77^b	*
o-Xylene	29.60 ± 1.88^{ab}	27.30 ± 3.02^a	37.21 ± 2.31^b	*	27.38 ± 2.00^{ab}	25.44 ± 2.36^a	34.03 ± 2.20^b	*
m-Xylene	37.15 ± 2.72^{ab}	35.03 ± 4.20^a	48.28 ± 3.75^b	*	35.47 ± 2.93^{ab}	33.25 ± 3.27^a	46.10 ± 3.30^b	*
p-Xylene	7.55 ± 0.86^{ab}	7.43 ± 1.37^a	11.60 ± 1.15^b	*	8.14 ± 1.06^{ab}	7.67 ± 1.09^a	11.65 ± 1.05^b	*
1-Phenyl-propane	4.73 ± 1.30^a	4.34 ± 1.20^a	6.42 ± 1.59^b	**	3.42 ± 0.97^a	3.30 ± 1.07^a	3.76 ± 1.07^a	ns
Carbon disulfide	238.26 ± 24.39^a	228.50 ± 13.81^a	213.91 ± 19.62^a	ns	247.90 ± 20.03^b	192.27 ± 15.16^{ab}	188.46 ± 15.59^a	*
5-Ethyldihydro-2(3H)-furanone	58.24 ± 1.72^b	59.08 ± 1.41^b	52.11 ± 2.44^a	*	59.11 ± 2.94^a	55.04 ± 2.34^a	51.35 ± 2.00^a	ns
5-Butyldihydro-2(3H)-furanone	7.51 ± 0.46^b	7.54 ± 0.32^b	6.15 ± 0.36^a	*	7.18 ± 0.59^a	7.03 ± 0.43^a	6.24 ± 0.46^a	ns

[1] Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. ^{a,b} Means bearing different superscript differ significantly ($P < 0.05$).
[2] Statistical significance in the analysis of variance: ns, non-significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 3
Levels[1] of the 2 volatile compounds significantly influenced by salt concentration in untreated or HPP-treated Iberian ham.

Compound	Untreated ham				HPP-treated ham			
	Low salt (n = 11) < 3.93%	Medium salt (n = 10) 3.93–4.60%	High salt (n = 9) > 4.60%	P^2	Low salt (n = 11) < 3.93%	Medium salt (n = 10) 3.93–4.60%	High salt (n = 9) > 4.60%	P^2
Ethanal	21.58 ± 4.20^b	10.70 ± 1.00^a	11.99 ± 2.26^{ab}	*	15.90 ± 2.99^a	9.40 ± 0.79^a	10.36 ± 2.07^a	ns
2,3-Pentanedione	6.14 ± 0.63^b	4.25 ± 0.16^a	4.85 ± 0.56^{ab}	*	4.61 ± 0.38^a	4.52 ± 0.33^a	3.96 ± 0.66^a	ns

[1] Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. ^{a,b} Means bearing different superscript differ significantly ($P < 0.05$).
[2] Statistical significance in the analysis of variance: ns, non-significant.
* $P < 0.05$.

Table 4
Levels[1] of the 5 volatile compounds significantly influenced by water activity (a_w) in untreated or HPP-treated Iberian ham.

Compound	Untreated ham				HPP-treated ham			
	Low a_w (n = 6) < 0.868	Medium a_w (n = 17) 0.868–0.883	High a_w (n = 7) > 0.883	P^2	Low a_w (n = 6) < 0.868	Medium a_w (n = 17) 0.868–0.883	High a_w (n = 7) > 0.883	P^2
Ethanol	487.89 ± 140.29^a	789.88 ± 97.14^{ab}	1134.57 ± 184.78^b	*	513.43 ± 161.22^a	813.58 ± 107.37^{ab}	1117.02 ± 163.67^b	*
2-Methylpropanal	70.26 ± 6.80^{ab}	81.61 ± 4.52^b	59.42 ± 5.39^a	*	65.15 ± 6.42^a	69.22 ± 5.42^a	49.99 ± 5.71^a	ns
Methanethiol	16.37 ± 1.34^a	15.88 ± 0.91^a	16.88 ± 1.53^a	ns	27.43 ± 2.97^a	32.74 ± 1.14^{ab}	35.02 ± 1.90^b	*
Dimethyl disulfide	151.50 ± 30.17^a	126.11 ± 10.08^a	143.72 ± 17.76^a	ns	129.17 ± 29.55^b	83.31 ± 8.54^{ab}	67.20 ± 11.46^a	*
Dimethyl trisulfide	35.54 ± 6.94^a	25.60 ± 3.43^a	30.96 ± 4.65^a	ns	76.47 ± 15.25^b	49.99 ± 4.39^{ab}	39.39 ± 8.10^a	*

[1] Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. ^{a,b} Means bearing different superscript differ significantly ($P < 0.05$).
[2] Statistical significance in the analysis of variance: ns, non-significant.
* $P < 0.05$.

relevant for its aroma. By means of GC-olfactometry, 28 volatile compounds (11 aldehydes, 7 sulfur compounds, 5 ketones, 2 nitrogen compounds, 2 esters, and 1 alcohol) were found to be odor-active, with differences between the odorants in intermuscular fat and lean (Carrapiso & García, 2004; Carrapiso, Ventanas, & García, 2002). In the present study, only 13 (7 aldehydes, 2 sulfur compounds, 2 ketones, 1 ester, and 1 alcohol) of those 28 odor-active compounds were detected.

3.2. Influence of intramuscular fat content

Intramuscular fat content of Iberian hams ranged from 4.63% to 18.59%, with a mean value of 11.96% (SD, 3.56%). Hams were grouped into 9 low fat hams (< 10.18%), 10 medium fat hams (10.18–13.75%) and 11 high fat hams (> 13.75%). The levels of 20 volatile compounds were significantly influenced by intramuscular fat content, 8 only in untreated control Iberian ham, 6 only in HPP-treated Iberian ham, and

Table 5
Levels[1] of the 35 volatile compounds significantly influenced by high pressure processing (HPP) of Iberian ham.

Compound	Untreated ham (n = 30)	HPP-treated ham (n = 30)	P[2]
Acetic acid	630.74 ± 19.16^{b}	561.65 ± 21.11^{a}	***
Propanoic acid	21.42 ± 0.68^{b}	19.00 ± 0.73^{a}	***
Butanoic acid	229.27 ± 9.69^{b}	202.28 ± 9.05^{a}	***
2-Methyl propanoic acid	81.78 ± 4.29^{b}	72.70 ± 3.65^{a}	***
2-Methyl butanoic acid	52.97 ± 2.78^{b}	48.25 ± 3.04^{a}	*
3-Methyl butanoic acid	100.49 ± 5.27^{b}	90.74 ± 5.89^{a}	**
Pentanoic acid	60.77 ± 2.57^{b}	54.08 ± 2.97^{a}	**
Hexanoic acid	335.53 ± 13.98^{b}	304.83 ± 16.93^{a}	*
Octanoic acid	18.25 ± 0.74^{b}	16.20 ± 0.80^{a}	**
2-Pentanol	80.11 ± 4.69^{b}	59.80 ± 5.10^{a}	***
Ethanal	15.08 ± 1.90^{b}	12.07 ± 1.36^{a}	***
2-Methylpropanal	74.16 ± 3.51^{b}	63.92 ± 3.78^{a}	***
2-Methylbutanal	344.31 ± 11.50^{b}	310.46 ± 12.61^{a}	***
3-Methylbutanal	461.80 ± 22.52^{b}	374.12 ± 18.45^{a}	***
2-Methyl-2-butenal	5.07 ± 0.21^{b}	4.39 ± 0.19^{a}	***
Pentanal	59.82 ± 3.14^{b}	53.11 ± 3.48^{a}	*
Nonanal	27.65 ± 1.47^{a}	34.77 ± 3.12^{b}	*
3-Methylthiopropanal	30.77 ± 2.88^{b}	24.14 ± 1.72^{a}	***
Ethyl propanoate	5.07 ± 0.68^{b}	4.49 ± 0.62^{a}	***
Ethyl-2-hydroxy-propanoate	11.58 ± 1.41^{b}	10.53 ± 1.27^{a}	*
Ethyl butanoate	11.19 ± 1.61^{b}	10.19 ± 1.45^{a}	*
Hexadecane	1.51 ± 0.08^{a}	1.63 ± 0.09^{b}	*
Branched chain alkane I	13.61 ± 0.89^{b}	9.26 ± 0.67^{a}	***
Branched chain alkane II	10.35 ± 0.66^{b}	7.21 ± 0.49^{a}	***
Cyclohexane	62.15 ± 5.71^{b}	41.32 ± 4.79^{a}	***
Ethylbenzene	128.75 ± 5.65^{b}	88.83 ± 4.30^{a}	***
1-Methylethylbenzene	21.52 ± 0.32^{b}	12.95 ± 0.17^{a}	***
Styrene	560.11 ± 60.53^{b}	342.03 ± 24.61^{a}	***
o-Xylene	31.62 ± 1.60^{b}	29.17 ± 1.42^{a}	*
Phenylethanal	94.61 ± 10.09^{b}	82.09 ± 9.99^{a}	***
1-Phenyl-propane	5.22 ± 0.90^{b}	3.51 ± 0.64^{a}	***
Methanethiol	16.21 ± 0.66^{a}	32.21 ± 1.06^{b}	***
Dimethyl sulfide	7.41 ± 0.72^{b}	4.55 ± 0.33^{a}	***
Dimethyl disulfide	135.29 ± 9.08^{b}	88.72 ± 8.65^{a}	***
Dimethyl trisulfide	28.84 ± 2.63^{a}	52.82 ± 4.76^{b}	***

[1] Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. ^{a,b} Means bearing different superscript differ significantly ($P < 0.05$).
[2] Statistical significance in the analysis of variance: ns, non-significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

6 in both untreated and HPP-treated Iberian ham (Table 2). In Serrano ham, 28 individual volatile compounds were affected by its intramuscular fat content, which had a mean value of 5.28% (Martínez-Onandi et al., 2016).

Information on the effect of intramuscular fat content on the volatile fraction of Iberian ham is scarce. Significant differences between the levels in muscle and adipose tissue of Iberian ham were found for 22 volatile compounds, which included 8 hydrocarbons, 4 aldehydes, 3 ketones, 5 ethers, 1 nitrogen compound and 1 sulfur compound, out of a total of 109 compounds (Narváez-Rivas et al., 2010).

Hexanoic acid was the only carboxylic acid significantly influenced by intramuscular fat content in untreated hams, with higher levels in low and medium fat content hams, while the levels of acetic, propanoic, butanoic and octanoic acids were influenced in HPP-treated hams (Table 2). Most carboxylic acids present in dry-cured ham are formed through lipid oxidation reactions, although acetic acid may also be formed by microbial fermentation of carbohydrates and Maillard reactions (Ramírez & Cava, 2007). No significant differences between the levels of carboxylic acids in muscle and adipose tissue of Iberian ham were found (Narváez-Rivas et al., 2010). There were no carboxylic acids among the odor-active compounds of Iberian ham (Carrapiso et al., 2002), but acetic, propanoic, butanoic and pentanoic acids were reported as odor-active compounds in spoiled bone-tainted Iberian ham (Carrapiso, Martín, Jurado, & García, 2010).

The only alcohol influenced by intramuscular fat content was pentanol, which reached significantly higher levels in untreated hams of low and medium fat content than in those of high fat content (Table 2). This alcohol was assigned pungent, strong, balsamic odor notes by GC-olfactometry (García-González, Tena, Aparicio-Ruiz, & Morales, 2008). Linear alcohols generally result from the degradation of lipid hydroperoxides (Ramirez & Cava, 2007). Their odor threshold values are usually high and their influence on the aroma is therefore low, not being included among the odor-active compounds of Iberian ham (Carrapiso et al., 2002, 2010).

Octanal was the only aldehyde influenced by intramuscular fat content, with significant differences between HPP-treated hams (Table 2). Linear aldehydes are products of lipid oxidation with low odor threshold values, which play an important role in the flavor of dry-cured ham. Octanal derives from oleic acid and may contribute to the aroma of Iberian ham with meat-like, green, fresh (García-González et al., 2008) or grass, fruity notes (Carrapiso et al., 2010).

The two ketones, 1-(2-furanyl)-ethanone and 3-ethylcyclopentanone, influenced by intramuscular fat content generally showed higher

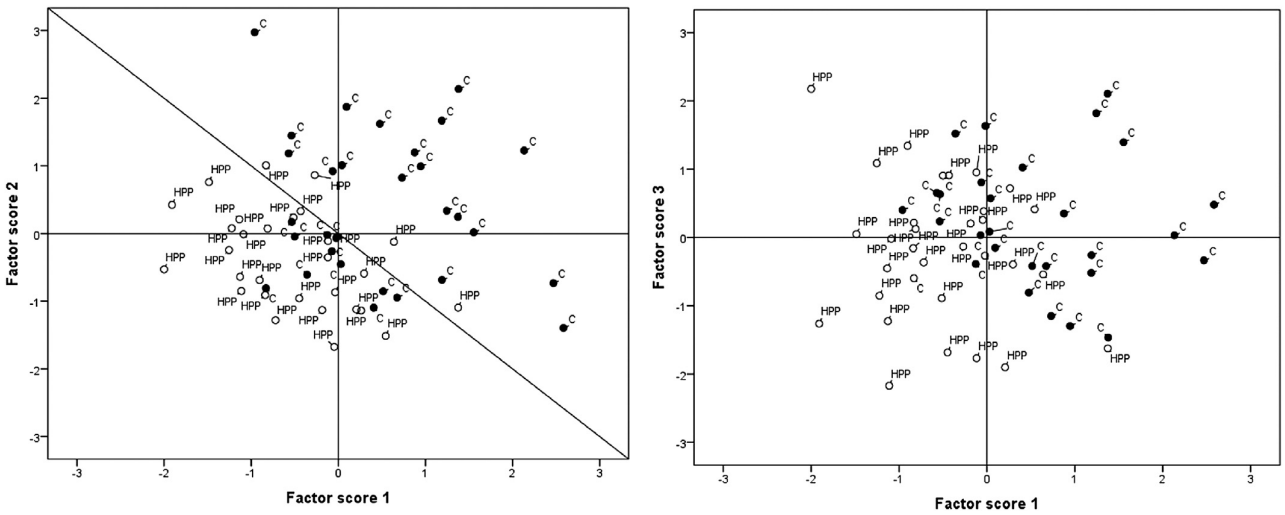


Fig. 1. Distribution of HPP-treated (HPP) and control (C) samples of Iberian ham according to the first three components (factor scores) of the PCA carried out on 9 selected volatile compounds significantly influenced by HPP treatment.

levels in low and medium fat content hams (Table 2). 3-Ethylcyclopentanone had been found in muscle and adipose tissue of Iberian ham (Narváez-Rivas et al., 2010), but none of these two ketones were among the odor-active compounds of Iberian ham (Carrapiso et al., 2002, 2010). Ketones may be formed through the chemical autooxidation of free fatty acids (Ramírez & Cava, 2007).

Benzene and benzaldehyde were influenced by intramuscular fat content, with higher levels in untreated hams of low fat content (Table 2). Benzene, with a sweet, aromatic, gasoline-like odor, has been occasionally detected in Iberian ham (Ruiz et al., 1999) while benzaldehyde, with bitter almonds, acorn odor notes, has been frequently found in Iberian ham (Narváez-Rivas et al., 2012) and was included among its odor-active compounds (García-González et al., 2008). Ethylphenol, not regarded as an odor-active compound in Iberian ham, showed higher levels in low and medium fat content hams than in high fat content hams (Table 2). In contrast, *m*-ethyltoluene, *o*-xylene, *m*-xylene and *p*-xylene, reached higher levels in high fat content hams (Table 2). Xylenes were correlated with Serrano dry-cured ham sensory characteristics, with *o*-xylene showing sweet-fruit and candy notes while *m*- and *p*-xylene had smoked-phenolic flavors (Flores, Grimm, Toldrá, & Spanier, 1997). The formation of alkylbenzenes from lipids and derived compounds throughout the long ripening period of Iberian ham, at temperatures close to 20 °C, was probably favored in ham of high fat content in comparison with ham of low fat content due to the greater availability of substrate. The concentration of xylenes suffered slight changes during the ripening period of Iberian ham, while methylbenzene decreased by 82% and ethylbenzene increased 18-fold from raw ham to 34-month ham (Narváez-Rivas, Gallardo, & León-Camacho, 2015). Alkylbenzenes were not considered to be odor-active compounds of Iberian ham (Carrapiso et al., 2002, 2010). However, methylbenzene and ethylbenzene, with plastic, glue, unpleasant odor notes (García-González et al., 2008), were associated with the volatile profiles of different dry-cured hams (Sánchez-Peña et al., 2005).

Carbon disulfide was the only sulfur compound influenced by intramuscular fat content, with higher levels in low fat content hams, although the differences were only significant in the case of HPP-treated hams (Table 2). This compound, with an ether-like and rotten egg odor, was detected in muscle and adipose tissue of Iberian ham (Narváez-Rivas et al., 2010). A less fat rich substrate probably favored the reactions responsible for the formation of sulfur volatile compounds, which mostly derive from sulfur-containing amino acids.

None of the four furans detected in Iberian ham was influenced by intramuscular fat content (Table 2). Furans are important contributors to the desirable aroma of dry-cured meat products because of their very low threshold value and their pleasant aroma (Ramírez & Cava, 2007). In fact, 2-ethylfuran and 2-pentyl-furane were included among the odor-active compounds of Iberian ham, with sweet, green, fruity notes (García-González et al., 2008).

In contrast, the levels of two furanones, 5-ethyldihydro-2(3H)-furanone and 5-butyldihydro-2(3H)-furanone, were influenced by intramuscular fat content (Table 2). Their highest levels were found in low and medium fat content hams, with significant differences in the case of untreated hams. Both furanones had been reported in Iberian ham (Ruiz et al., 1998). In agreement with our results, 5-ethyldihydro-2(3H)-furanone was at 4.25-fold higher levels in muscle than in adipose tissue of Iberian ham (Narváez-Rivas et al., 2010). Furanones may be formed in ham through Maillard reactions and some of them impart pleasant odors such as cocoa, butter or fruit (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004).

None of the pyrazines or the miscellaneous compounds detected in the present study was significantly influenced by the intramuscular fat content of Iberian ham (Table 2).

3.3. Influence of salt concentration and water activity

The NaCl concentration in Iberian hams ranged from 3.02% to 5.62%, with a mean value of 4.27% (SD, 0.67%). Iberian hams were grouped into 11 hams of low (< 3.93%), 10 of medium (3.93–4.60%) and 9 of high (> 4.60%) NaCl content. The levels of 2 volatile compounds were significantly influenced by salt concentration, but only in untreated samples (Table 3). Low salt hams had the highest levels of ethanal and 2,3-pentanodione, compounds derived from lipid oxidation. The same trend was observed for HPP-treated samples, although the differences were not significant. Salt concentration of Iberian ham caused small differences in lipid oxidative changes which depended on the muscle, with hexanal reaching higher levels in the *Biceps femoris* muscle and lower levels in the *Semimembranosus* muscle of high salt hams than in the respective muscles of low salt hams (Andrés et al., 2004a). According to the same authors, salt concentration of Iberian ham did not influence the levels of branched-chain aldehydes or ketones, with 2-pentylfuran as the only volatile compound significantly influenced by salt (Andrés et al., 2007). The effect of salt concentration on volatile compounds was less marked in Iberian ham than in Serrano ham, in which 23 individual volatile compounds were affected (Martínez-Onandi et al., 2016), probably because of the higher NaCl ranges in Serrano ham (2.87% to 7.91% NaCl) than in Iberian ham (3.02% to 5.62% NaCl).

The salt-in-lean ratio of Iberian hams ranged from 0.036 to 0.063, with a mean value of 0.049 (SD, 0.008). They were grouped into 12 hams of low (< 0.045), 9 of medium (0.045–0.052) and 9 of high (> 0.052) salt-in-lean ratio. The salt-in-lean ratio did not significantly influence the levels of any of the volatile compounds in untreated or HPP-treated samples. Contrarily, 18 individual volatile compounds were affected in Serrano ham (Martínez-Onandi et al., 2016), in which the salt-in-lean ratio ranged from 0.033 to 0.081.

The water activity (a_w) of Iberian hams ranged from 0.845 to 0.912, with a mean value of 0.876 (SD, 0.015). They were grouped into 6 low a_w (< 0.868), 17 medium a_w (0.868–0.883) and 7 high a_w (> 0.883) hams. The levels of 2 volatile compounds in untreated control samples and 4 volatile compounds in HPP-treated samples were significantly influenced by a_w (Table 4). Thirteen individual volatile compounds were affected in Serrano ham (Martínez-Onandi et al., 2016), in which a_w values ranged from 0.833 to 0.883. In Iberian hams, low a_w values decreased the formation of ethanol while they enhanced the formation of 2-methylpropanal, dimethyl disulfide and dimethyl trisulfide (Table 4). Ethanol most probably originates from microbial metabolism, 2-methylpropanal from the Strecker degradation of valine, and dimethyl disulfide and dimethyl trisulfide from Maillard reactions (Ramírez & Cava, 2007). Enzymatic activity in dry-cured ham was influenced by the decrease in a_w (Toldrá, Flores, & Sanz, 1997). Low a_w values were shown to hinder the activity of muscle protease and aminopeptidases (Toldrá, Rico, & Flores, 1992; Toldrá, Aristoy, & Flores, 2000), what should result in lower concentrations of free amino acids, while they hardly affected the activity of neutral and basic muscle lipases (Motilva & Toldrá, 1993). The higher levels of some volatile compounds derived from free amino acids such as 2-methylpropanal, dimethyl disulfide and dimethyl trisulfide in Iberian ham of low a_w values (Table 4) might be due to an enhancement of the reactions involved in amino acid catabolism under those conditions. 2-Methylpropanal, with pungent and nutty odor notes, dimethyl trisulfide, with rotten eggs and burnt odor notes, and methanethiol, with cabbage and rotten eggs odor notes, were among the odor-active compounds of Iberian ham (Carrapiso et al., 2002).

3.4. Effect of high pressure processing

The levels of 35 out of the 122 volatile compounds found in Iberian ham varied significantly with HPP treatment. Four compounds reached higher levels in HPP-treated samples and 31 compounds in untreated

samples (Table 5). In a previous work on the effect of HPP on the volatile fraction of Iberian ham, 5 aldehydes increased and 1 aldehyde decreased after treatment at 600 MPa for 6 min at 12 °C (Fuentes et al., 2010). In Serrano ham 4 volatile compounds increased and 26 decreased after treatment at 400 MPa for 10 min at 12 °C (Rivas-Cañedo et al., 2009), while 2 volatile compounds increased and 6 decreased after treatment at 600 MPa for 6 min at 21 °C (Martínez-Onandi et al., 2016).

In the present study, the levels of nonanal, derived from lipid oxidation, hexadecane, probably coming from animal feeding, and methanethiol and dimethyl trisulfide, which might result from the reaction of cysteine and methionine with carboxylic compounds like 2,3-pentadione derived from lipid oxidation (Muriel et al., 2004), were significantly higher in HPP-treated Iberian ham than in control ham (Table 5). In contrast, the levels of 6 linear and 3 branched-chain carboxylic acids, derived from lipid oxidation or microbial metabolism, 2-pentanol, generated through the oxidation of polyunsaturated fatty acids, 2 linear and 5 branched-chain aldehydes, most probably coming from Strecker degradation, 3 esters, of microbial or unknown origin, 2 branched-chain and 1 cyclic alkane, presumably coming from animal feeding, 6 benzenic compounds, generated by the cyclization of long chain unsaturated hydrocarbons, and 2 sulfur compounds, formed through the reaction of sulfur-containing amino acids with carboxylic compounds, were at significantly lower levels in HPP-treated Iberian ham than in control ham (Table 5).

Total levels of volatile compounds derived from lipid oxidation declined in HPP-treated samples by 3.7%, total levels of volatile compounds generated through Maillard reactions by 9.0% and total levels of volatile compounds of microbial or unknown origin by 9.7%, in comparison with untreated samples. According to the analysis of variance, significant declines in the total levels of chemical groups of volatile compounds after HPP treatment of Iberian ham were found only for carboxylic acids, with a 10.5% decrease ($P < 0.001$), aldehydes, with a 1.2% decrease ($P < 0.01$), and benzene compounds, with a 12.8% decrease ($P < 0.001$).

The first three components of the PCA carried out on the total levels of 11 groups of volatile compounds in HPP-treated and untreated Iberian ham samples jointly explained 60.3% of the variance while the first three components of the PCA carried out on only 7 selected groups of volatile compounds jointly explained 84.0% of the variance. Component 1, including furanes and alkanes, explained 34.7% of the variance, component 2, including ketones, aldehydes and furanones, explained 27.1% of the variance, and component 3, including esters and alcohols, explained 22.2% of the variance. However, the separation of HPP-treated from control samples of Iberian ham was not feasible by means of this PCA (data not shown).

In the PCA carried out on the 35 individual volatile compounds influenced by HPP treatment, the first three components jointly explained only 49.5% of the variance (data not shown). However, the first three components of the PCA carried out on 9 selected volatile compounds jointly explained 88.5% of the variance. Component 1, including 1-phenyl-propane, ethylbenzene, cyclohexane, branched chain alkane I and branched chain alkane II, explained 52.2% of the variance, component 2, including dimethyl sulfide and 2-methyl-2-butenal, explained 20.8% of the variance, and component 3, including propanoic acid and acetic acid, explained 15.5% of the variance. Factor scores 1 and 2 of this PCA separated control samples of Iberian ham, mostly located above the bisectrix of the second and fourth quadrants, from HPP-treated samples, mostly located below that bisectrix (Fig. 1). Factor scores 1 and 3 of this PCA separated control samples of Iberian ham, mostly located in the first and fourth quadrants, from HPP-treated samples, mostly located in the second and third quadrants.

Radical formation increases and lipid oxidation reactions are promoted in HPP-treated dry-cured ham, in particular when 600 MPa are applied (Andrés et al., 2004b; Fuentes et al., 2010). In Serrano ham packaged in multi-layer plastic material, higher levels of 4 volatile compounds and lower levels of 26 individual volatile compounds, including 14 branched-chain alkanes, were found after treatment at 400 MPa (Rivas-Cañedo et al., 2009). In Serrano ham treated at 600 MPa, the levels of 2 sulfur compounds increased while those of 4 esters and 2 sulfur compounds decreased (Martínez-Onandi et al., 2016). In Iberian ham treated at 600 MPa, the levels of five aldehydes and the perception of rancid odor increased while the levels of two aldehydes decreased (Fuentes et al., 2010). Dry-cured ham sensory characteristics may become altered due to the chemical changes induced by HPP treatment (Clariana et al., 2011; Lorido, Estévez, Ventanas, & Ventanas, 2015),

Volatile compounds derived from microbial metabolism seem to be particularly affected by HPP, a treatment which causes microbial death and decreases metabolic rates. HPP treatment of dry-cured meat products also decreases the activity of muscle enzymes such as catepsins, aminopeptidases and dipeptidylpeptidases (Campus, Flores, Martinez, & Toldrá, 2008), which might influence the formation of volatile compounds. Independently of the origin of compounds, the effect of HPP treatment on the volatile fraction of dry-cured meat products primarily depends on the type of product and the pressure level, although the influence of other factors such as temperature and time of HPP treatment, packaging material and product storage conditions should not be neglected.

4. Conclusions

It can be concluded that intramuscular fat content was the physicochemical characteristic with the greatest influence on Iberian ham volatile fraction. A fat-richer substrate apparently favored the formation of volatile compounds derived from lipid oxidation, which represented around 75% of Iberian ham volatile fraction. Other physicochemical characteristics of Iberian ham such as salt concentration, salt-in-lean ratio and water activity only affected the levels of 2, 0 and 5 volatile compounds, respectively. It can also be concluded that HPP treatment of Iberian ham altered its volatile fraction, with an increase in the levels of 4 individual compounds, a decrease in the levels of 31 individual compounds, and significant decreases in the total levels of chemical groups such as carboxylic acids, aldehydes and benzene compounds. The higher levels of nonanal, methanethiol and dimethyl trisulfide, with unpleasant odor notes, together with the lower levels of odor-active volatile compounds with pleasant odor notes, might alter the sensory characteristics of Iberian ham when submitted to HPP treatment at 600 MPa. The conditions for HPP treatment of Iberian ham should be established taking into account its possible effect on odor and aroma characteristics.

Acknowledgments

The authors wish to thank E. Fulladosa, P. Gou and J. Arnau (IRTA, Monells, Spain) for the selection and processing of hams, the Spanish Ministry of Science and Innovation for project RTA2010-00029-C04-04 which supported the experimental work, and INIA for funding the Ph.D. grant of N. Martínez-Onandi.

References

Andrés, A. I., Cava, R., Ventanas, J., Muriel, E., & Ruiz, J. (2004a). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, *84*, 375–381.

Andrés, A. I., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2004b). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology*, *219*, 205–210.

Andrés, A. I., Cava, R., Martin, D., Ventanas, J., & Ruiz, J. (2005). Lipolysis in dry-cured ham: Influence of salt content and processing conditions. *Food Chemistry*, *90*, 523–533.

Andrés, A. I., Adamsen, C. E., Møller, J. K. S., Ruiz, J., & Skibsted, L. H. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research and*

Technology, *222*, 486–491.
Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2007). Effect of salt content and processing conditions on volatile compounds formation throughout the ripening of Iberian ham. *European Food Research and Technology*, *225*, 677–684.
AOAC (2000). *Official methods of analysis*(17th ed.). Gaithersburg, Maryland, USA: Association of Official Analytical Chemists.
Armenteros, M., Toldrá, F., Aristoy, M. C., Ventanas, J., & Estévez, M. (2012). Effect of the partial replacement of sodium chloride by other salts on the formation of volatile compounds during ripening of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *60*, 7607–7615.
Blesa, E., Aliño, M., Barat, J. M., Grau, R., Toldrá, F., & Pagán, M. J. (2008). Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, *78*, 135–142.
Campus, M., Flores, M., Martinez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, *80*, 1174–1181.
Carrapiso, A. I., & García, C. (2004). Iberian ham headspace: Odorants of intermuscular fat and differences with lean. *Journal of the Science of Food and Agriculture*, *84*, 2047–2051.
Carrapiso, A. I., Ventanas, J., & García, C. (2002). Characterization of the most odor-active compounds of Iberian ham headspace. *Journal of Agricultural and Food Chemistry*, *50*, 1996–2000.
Carrapiso, A. I., Martín, L., Jurado, A., & García, C. (2010). Characterization of the most odour-active compounds of bone tainted dry-cured Iberian ham. *Meat Science*, *85*, 54–58.
Carrapiso, A. I., Noseda, B., García, C., Reina, R., Sánchez del Pulgar, J., & Devlieghere, F. (2015). SIFT-MS analysis of Iberian hams from pigs reared under different conditions. *Meat Science*, *104*, 8–13.
Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, A., & García-Regueiro, J. A. (2011). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Science & Emerging Technologies*, *12*, 456–465.
Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry*, *45*, 2178–2186.
Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method from the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.
Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, *85*, 506–514.
García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry*, *41*, 23–32.
García-González, D. L., Tena, N., Aparicio-Ruiz, R., & Morales, M. T. (2008). Relationship between sensory attributes and volatile compounds qualifying dry-cured hams. *Meat Science*, *80*, 315–325.
Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science & Emerging Technologies*, *5*, 451–457.
López, M. O., de la Hoz, L., Cambero, M. I., Gallardo, E., Reglero, G., & Ordóñez, J. A. (1992). Volatile compounds of dry hams from Iberian pigs. *Meat Science*, *31*, 267–277.
Lorido, L., Estévez, M., Ventanas, J., & Ventanas, S. (2015). Comparative study between Serrano and Iberian dry-cured hams in relation to the application of high hydrostatic pressure and temporal sensory perceptions. *LWT- Food Science and Technology*, *64*, 1234–1242.
Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., & Picon, A. (2016). Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. *Meat Science*, *111*, 130–138.
Motilva, M.-J., & Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *196*, 228–232.
Muriel, E., Antequera, T., Petrón, M. J., Andrés, A. I., & Ruiz, J. (2004). Volatile compounds in Iberian dry-cured loin. *Meat Science*, *68*, 391–400.
Narváez-Rivas, M., Gallardo, E., Ríos, J. J., & León-Camacho, M. (2010). A tentative characterization of volatile compounds from Iberian dry-cured ham according to different anatomical locations. A detailed study. *Grasas y Aceites*, *61*, 369–377.
Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: A review. *Grasas y Aceites*, *63*, 432–454.
Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2015). Evolution of volatile hydrocarbons from subcutaneous fat during ripening of Iberian dry-cured ham. A tool to differentiate between ripening periods of the process. *Food Research International*, *67*, 299–307.
Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry*, *55*, 1923–1931.
Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing; effect of the packaging material. *Meat Science*, *82*, 162–169.
Ruiz, J., Cava, R., Ventanas, J., & Jensen, M. T. (1998). Headspace solid phase microextraction for the analysis of volatiles in a meat product: Dry-cured Iberian ham. *Journal of Agricultural and Food Chemistry*, *46*, 4688–4694.
Ruiz, J., Ventanas, J., Cava, R., Andrés, A., & García, C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science*, *52*, 19–27.
Sabio, E., Vidal-Aragón, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chemistry*, *61*, 493–503.
Sánchez-Peña, C. M., Luna, G., García-González, D. L., & Aparicio, R. (2005). Characterization of French and Spanish dry-cured hams: Influence of the volatiles from the muscles and the subcutaneous fat quantified by SPME-GC. *Meat Science*, *69*, 635–645.
Santos-Garcés, E., Gou, P., García-Gil, N., Arnau, J., & Fulladosa, E. (2010). Non-destructive analysis of a_w, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, *101*, 187–192.
Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A., & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science*, *58*, 85–91.
Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science*, *38*, 331–352.
Toldrá, F., Rico, E., & Flores, J. (1992). Activity of pork muscle proteases in model cured meat systems. *Biochimie*, *74*, 291–296.
Toldrá, F., Flores, M., & Sanz, Y. (1997). Dry-cured ham flavour: Enzymatic generation and process influence. *Food Chemistry*, *59*, 523–530.
Toldrá, F., Aristoy, M.-C., & Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International*, *33*, 181–185.
Ventanas, S., Estevez, M., Andrés, A. I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME-DED. *Meat Science*, *79*, 172–180.
Wang, J. M., Jin, G. F., Zhang, W. G., Ahn, D. U., & Zhang, J. H. (2012). Effect of curing salt content on lipid oxidation and volatile flavour compounds of dry-cured turkey ham. *LWT- Food Science and Technology*, *48*, 102–106.
Zhang, J. H., Zhen, Z. Y., Zhang, W. G., Zeng, T., & Zhou, G. H. (2009). Effect of intensifying high-temperature ripening on proteolysis, lipolysis and flavor of Jinhua ham. *Journal of the Science of Food and Agriculture*, *89*, 834–842.
Zhang, J. H., Jin, G. F., Wang, J. M., & Zhang, W. G. (2011). Effect of intensifying high-temperature ripening on lipolysis and lipid oxidation of Jinhua ham. *LWT- Food Science and Technology*, *44*, 473–479.

6. Influence of physicochemical parameters and high pressure processing on the volatile compounds of Iberian dry-cured ham after prolonged refrigerated storage

Artículo enviado a *Meat Science*

Elsevier Editorial System(tm) for Meat Science

Manuscript Draft

Manuscript Number: MEATSCI-D-16-00213R1

Title: Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage

Article Type: Research Paper

Keywords: Serrano dry-cured ham; volatile compound; high pressure processing; water activity; salt; intramuscular fat.

Corresponding Author: Prof. Manuel Nunez, PhD

Corresponding Author's Institution: INIA

First Author: Nerea Martínez-Onandi, MSc

Order of Authors: Nerea Martínez-Onandi, MSc; Ana Rivas-Cañedo, PhD; Antonia Picon, PhD; Manuel Nunez, PhD

ABSTRACT

The effect of high pressure processing (HPP) at 600 MPa and refrigerated storage for 5 months on the volatile fraction of 30 Iberian dry-cured hams of different compositional characteristics was investigated. A total number of 116 volatile compounds were identified in Iberian ham. Its compositional characteristics significantly influenced the levels of 11 volatile compounds (3 alcohols, 3 carboxylic acids, 2 alkanes, 2 benzene compounds and 1 aldehyde). HPP treatment had a significant effect on the levels of 34 volatile compounds, with higher levels of 11 compounds and lower levels of 23 compounds in HPP-treated samples than in control samples. During refrigerated storage of Iberian ham, the number of volatile compounds declined, the total abundance of volatile compounds decreased by 5.1 % in control ham, and the levels of 75 volatile compounds varied significantly. HPP treatment conditions and commercial shelf life length should be established in order to maintain the unique odor and aroma characteristics of Iberian ham.

Keywords

Iberian ham, Volatile compound, Intramuscular fat, Salt, High pressure processing, Shelf life

1. Introduction

Iberian ham is a Spanish dry-cured meat product obtained following a traditional manufacturing process, which usually lasts 2 to 4 years. Hind legs of Iberian breed pigs previously rubbed with salt, with or without nitrates and nitrites, are disposed in layers with abundant salt in between at low (1 - 3 ºC) temperature and high (90 - 95%) relative humidity (RH) for 9 to 12 days. Afterwards, during the post-salting stage, salt is allowed to diffuse into the ham interior for several months at slightly higher temperatures and lower RH levels. Finally, hams are ripened for a period of at least 18 months during which the temperature is gradually risen up to 25 ºC or above and the RH simultaneously lowered down to 65% (Andrés, Cava, Martin, Ventanas, & Ruiz, 2005). This traditional manufacturing process allows the development of the unique sensory characteristics of Iberian ham and achieves a microbiologically safe product (Rodríguez, Nuñez, Córdoba, Bermúdez, & Asensio, 1996).

Primary reactions related to proteolysis and lipolysis during the manufacture and ripening of the different types of dry-cured ham generate small peptides, free amino acids and free fatty acids (Toldrá & Flores, 1998; Toldrá, 2006; Zhang, Zeng, Zhang, Zeng, & Zhou, 2009). Through further secondary reactions taking place mostly during the ripening period, part of the hydrolysis products are transformed into the compounds responsible for the odor, aroma and flavor of dry-cured ham (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2007; Zhou & Zhao, 2007). Many of those derived compounds are present in the volatile fraction of dry-cured ham.

Some of the compositional characteristics of dry-cured ham, such as intramuscular fat content, derive from the properties of the raw material and are related to breed, sex and age of the animal, diet and type of management. Others, such as salt concentration and water activity (a_w), depend upon manufacturing procedures including *post-mortem* stage, salting time and ripening conditions.

The intramuscular fat of dry-cured ham is the main precursor of lipid-derived compounds (Motilva, Toldrá, Nieto, & Flores, 1993; Carrapiso & García, 2004; Carrapiso, Noseda, García, Reina, Sánchez del Pulgar, & Devlieghere, 2015) while salt concentration and a_w influence the solubilization of myofibrillar proteins and the activity of proteolytic and lipolytic enzymes (Motilva et al., 1993; Toldrá & Flores, 1998; Andrés et al., 2005).

Salting is a crucial step in the manufacturing process of dry-cured ham, albeit an excess of salt intake has been associated with a higher prevalence of cardiovascular diseases. In addition, low-fat food products are rapidly gaining acceptance in many countries in order to control body weight. To satisfy these health-oriented consumer demands, the meat industry tends to produce dry-cured hams with lower salt and fat contents (Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Casabianca, 1999; Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004a; Armenteros, Toldrá, Aristoy, Ventanas, & Estévez, 2012). However, variations in the salt concentration and the intramuscular fat content of Iberian ham might impair its unique sensory properties, in particular aroma and flavor (Andrés et al., 2007; Ventanas, Estevez, Andrés, & Ruiz, 2008; Armenteros et al., 2012; Carrapiso et al., 2015).

Another consumer demand regarding dry-cured ham is the ready-to-eat presentation, which implies deboning and slicing followed by vacuum or modified atmosphere packaging (García-Esteban, Ansorena, & Astiasarán, 2004; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004). These additional manufacturing practices may result in the contamination of ham slices by spoilage microorganisms or even pathogens such as *Listeria monocytogenes*. In order to guarantee the microbiological stability and safety of the product throughout its shelf life, high pressure processing (HPP) is frequently applied to sliced dry-cured ham (Morales, Calzada, & Nuñez, 2006; Hereu, Bover-Cid, Garriga, & Aymerich, 2012). However, the effect of HPP on dry-cured ham constituents and enzymes may influence lipid oxidation (Andrés, Møller, Adamsen, & Skibsted, 2004b; Cava,

Ladero, González, Carrasco, & Ramírez, 2009) and volatile compounds (Rivas-Cañedo, Fernández-García, & Nuñez, 2009b; Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016a).

In addition, refrigerated storage of Iberian ham along its commercial shelf life after HPP treatment might alter the levels of aroma compounds, as shown for Serrano ham (Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016b). Although the effect of HPP on the volatile compounds of Iberian ham immediately after treatment has been investigated (Martínez-Onandi et al, 2017), there is no information about its effect on the evolvement of volatile compounds along its commercial shelf life under refrigerated storage conditions. To our knowledge, in this regard only the effect of HPP on the oxidative stability of Iberian ham treated at 200 and 400 MPa during storage for 39 days at 5 ºC has been reported (Andrés, Adamsen, Møller, Ruiz, & Skibsted, 2006).

The objective of the present study was to investigate the influence of compositional characteristics, HPP treatment and a prolonged refrigerated storage period, equivalent to commercial shelf life conditions, on the volatile fraction of Iberian ham.

2. Material and methods

2.1. Selection, sampling and HPP of Iberian hams

Thirty Iberian hams from 50 % Iberian x Duroc animals, manufactured and ripened at a processing plant in Extremadura (Spain) were used in the present study. Hams were selected on the basis of subcutaneous fat thickness and computed tomography-estimated salt concentration (Santos-Garcés, Gou, García-Gil, Arnau, & Fulladosa, 2010) at the Institute of Food and Agricultural Research (IRTA, Monells, Spain) facilities. Iberian hams were of similar characteristics and from the same lot of animals as those analyzed in an earlier work (Martínez-Onandi et al., 2017).

Two slices (150-200 g in weight) were obtained from the cushion of each ham and separately vacuum-packaged in polyamide + polyethylene bags (Mobepack, Salamanca, Spain). The first slice was HPP-treated at 600 MPa for 6 min at 21 ºC as previously described (Martínez-Onandi et al., 2016a) and the second slice served as control. Both slices were held for 5 months at 4 ºC, under time-temperature conditions similar to those of shelf life commercial storage. After this period, slices were kept at -35 ºC until analysis, which was carried out within 1 month.

2.2. Physicochemical and volatile compound analysis

A representative 100 g portion of each ham slice was minced by means of a mechanical grinder (IKA Labortechnik, Staufen, Germany) for physicochemical analysis. Intramuscular fat content, chloride concentration and a_w were determined as previously described (Martínez-Onandi et al., 2016a). All determinations were performed in triplicate.

Volatile compounds were extracted from representative minced portions of ham slices with added anhydrous Na_2SO_4 and cyclohexanone as internal standard by solid-phase microextraction (SPME). They were analyzed by gas chromatography-mass spectrometry (GC-MS) following a previously described procedure (Martínez-Onandi et al., 2016a). Injection of commercial standards (Sigma-Aldrich), spectra comparison using the Wiley7Nist05 Library (Wiley Spectra Lab, Weinheim, Germany) and/or calculation of linear retention indices (LRI) relative to a series of alkanes (C5-C20) were used for the identification of volatile compounds. Semi-quantitative determination of volatile compounds was performed by using the sums of the abundances of selected characteristic ions. The data obtained were multiplied by 10^{-5} to facilitate comprehension. Each of the ham slices was analyzed in triplicate.

2.3. Statistical analysis

SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA) was used for data analysis. Three groups of hams with low, medium and high values for each of the physicochemical characteristics were established by using the mean ± 0.5 standard deviations (SD) criterion previously described (Martínez-Onandi et al., 2016a). In the one-way analysis of variance (ANOVA) on the levels of volatile compounds with intramuscular fat content, salt concentration, salt-in-lean ratio (S/L) or a_w as the main effect, ham was a random effect. In the ANOVA with HPP treatment as the main effect, ham was a fixed effect. Tukey's test, with the significance assigned at $P < 0.05$, was used for the comparison of means between groups of hams. Principal component analysis (PCA), with Varimax rotation, was performed on the levels of individual volatile compounds, the total levels of chemical groups of volatile compounds, and the physicochemical characteristics, using the same statistical package.

3. Results and discussion

Table 1 lists the 116 volatile compounds detected in the volatile fraction of ripened Iberian ham after 5 months of refrigerated storage at 4 ºC grouped by chemical families, as well as their linear retention indexes, the ions used for quantification and the identification method. Volatile compounds found in Iberian ham after refrigerated storage included 11 carboxylic acids, 19 alcohols, 13 aldehydes, 16 ketones, 7 esters, 9 alkanes, 21 benzene compounds, 5 sulfur compounds, 4 furanes, 5 furanones, 3 pyrazines and 3 miscellaneous compounds.

3.1. Influence of physicochemical characteristics

Intramuscular fat content ranged from 8.87 % to 16.62%, with a mean value of 12.38 % (SD, 2.12 %). Hams were grouped into 10 low fat (< 11.32 %), 11 medium fat (11.32-13.44 %) and 9

high fat (> 13.44 %) hams. In control hams, intramuscular fat content influenced the levels of 2-pentanol, 2-hexanol and 2-phenylethanol, with higher levels in low fat hams, and of ethanal, with higher levels in high fat hams, while in HPP-treated hams it only influenced the level of pentane, with higher levels in high fat hams (Table 2). The influence of intramuscular fat content on the volatile compounds of Iberian ham was considerably less marked at the end than at the beginning of the refrigerated storage period, when the levels of 20 volatile compounds were influenced by this compositional characteristic (Martínez-Onandi et al., 2017). Alcohols such as 2-pentanol and 2-hexanol are generated through the oxidation of polyunsaturated fatty acids while linear aldehydes such as ethanal are products of lipid oxidation, hydrocarbons such as pentane come from the oxidative decomposition of lipids, and benzene compounds such as 2-phenylethanol are generated by the cyclization of long chain unsaturated hydrocarbons (Ramírez & Cava, 2007; Narváez-Rivas, Gallardo, & León-Camacho, 2012). In Iberian loins of different intramuscular fat content, a higher total chromatographic area and higher levels of some volatile compounds, derived from lipid oxidation such as hexanol, octanal, (E,E)-2,4-heptadienal or (E)-2-decenal or from amino acid degradation such as dimethylsulfide, 3-methylbutanal or phenylacetaldehyde, were detected in the headspace of high fat loins compared to low fat loins (Ventanas et al., 2008).

Salt concentration ranged from 3.51 % to 5.35 %, with a mean value of 4.48 % (SD, 0.50 %). Hams were grouped into 12 low salt (< 4.23 %), 7 medium salt (4.23-4.72 %) and 11 high salt (> 4.72 %) hams. In control hams, salt concentration influenced the levels of acetic, propanoic and nonanoic acids as well as phenylethanal, with higher levels in medium salt hams, while in HPP-treated hams, acetic acid, propanoic acid and 3-methyl-1-butanol were influenced by salt concentration, with patterns similar to those in control hams (Table 3). At the beginning of the refrigerated storage period, ethanal and 2,3 pentanedione were the only volatile compounds influenced by salt concentration (Martínez-Onandi et al., 2017). Acetic acid, propanoic acid and 3-

methyl-1-butanol may derive from microbial metabolism or from Maillard reactions while nonanoic acid most probably comes from lipid oxidation, and phenylethanal requires the previous cyclization of long chain unsaturated hydrocarbons (Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006; Ramírez & Cava, 2007; Narváez-Rivas et al., 2012). Variations in NaCl concentration and replacement of NaCl by other salts have been proven to influence the formation of some volatile compounds during ripening of dry-cured ham, probably by affecting lipid oxidation and proteolysis phenomena (Andrés at al., 2007; Armenteros et al., 2012; Wang, Jin, Zhang, Ahn, & Zhang, 2012).

The salt-in-lean ratio of hams ranged from 0.042 to 0.061, with a mean value of 0.051 (SD, 0.005). Hams were grouped into 10 low S/L (< 0.048), 9 medium S/L (0.048-0.054) and 11 high S/L (> 0.054) hams. In control hams, the S/L ratio influenced the levels of acetic acid, with higher levels in medium S/L hams, and ethanal, with higher levels in low S/L hams, while in HPP-treated hams the S/L ratio influenced the levels of acetic acid, with higher levels in medium S/L hams, and 3-methyl-1-butanol and cyclohexane, with higher levels in low S/L hams (Table 4). In contrast, the S/L ratio did not influence any of the volatile compounds of Iberian ham at the beginning of the refrigerated storage period (Martínez-Onandi et al., 2017). Acetic acid, ethanal and 3-methyl-1-butanol are mainly formed through microbial metabolic reactions which might be modulated by the S/L ratio of hams during refrigerated storage.

Water activity (a_w) ranged from 0.859 to 0.890, with a mean value of 0.875 (SD, 0.008). Hams were grouped into 11 low a_w (< 0.871), 9 medium a_w (0.871-0.879) and 10 high a_w (> 0.879) hams. In control hams, a_w influenced the levels of acetic acid, with higher levels in medium a_w hams, and ethanal, with higher levels in high a_w hams, while in HPP-treated hams a_w influenced the levels of acetic acid, with higher levels in medium a_w hams, and 3-methyl-1-butanol and cyclohexane, with higher levels in high a_w hams (Table 5). Low a_w hams were those of high S/L ratio and high a_w hams those of low S/L ratio. Consequently, compounds in Tables 4 and 5 were coincident and, as

expected, the effect of a_w on volatile compounds was exactly the opposite to the effect of S/L ratio. At the beginning of the refrigerated storage period of Iberian ham, volatile compounds influenced by a_w were ethanol, 2-methyl-propanal, methanethiol, dimethyl disulfide and dimethyl trisulfide (Martínez-Onandi et al., 2017), all of them different from those influenced after refrigerated storage. Water activity of dry-cured ham regulates the formation of volatile compounds through its effect on the activity of enzymes, which progressively decrease as a_w declines during ripening excepting acid lipase and acid esterase which remain unaltered (Toldrá, Flores, & Sanz, 1997; Toldrá, 2006).

3.2. Influence of high pressure processing

HPP treatment of Iberian ham significantly influenced the levels of 34 volatile compounds after refrigerated storage for 5 months at 4 ºC (Table 6). At that time, the sum of the levels of all the volatile compounds was on average only 1.2 % lower in HPP-treated ham than in control ham, which was not a statistically significant difference. Eleven volatile compounds (nonanoic acid, 1-penten-3-ol, 2-butoxyethanol, decanal, 2-butanone, 2-pentanone, 2-hexanone, methanethiol, carbon disulfide, dimethyl trisulfide and *p*-nitrophenyl hexanoate) reached higher levels in HPP-treated samples than in control samples (Table 6). Particularly, the level of dimethyl trisulfide was 4.4-fold higher in HPP-treated samples than in control samples after refrigerated storage, even though its level at the beginning of refrigerated storage was only 1.8-fold higher in HPP-treated samples (Martínez-Onandi et al., 2017).

Results obtained in previous studies on the effect of HPP on the volatile fraction of dry-cured ham differ. Thus, in Serrano ham analyzed shortly after HPP treatment, ethanal, 2-methyl-2-propenal, 2-heptanone, 1-octene and 1-undecene were the only volatile compounds reaching higher levels in HPP-treated samples than in control samples according to Rivas-Cañedo et al. (2009b) and methanethiol and sulfur dioxide according to Martínez-Onandi et al. (2016a) while in Serrano ham

analyzed after 5 months of refrigerated storage following HPP, 1-octanol, 1-penten-3-ol, 1-octen-3-ol, 2-propanone, 2-butanone, 2-pentanone, 5-methyl-3-heptanone, hexane, dodecane, 3-phenyl-2-propenal, methanethiol and sulfur dioxide reached higher levels in HPP-treated samples than in control samples (Martínez-Onandi et al., 2016b). In the case of Iberian ham analyzed at the beginning of the refrigerated storage period, nonanal, hexadecane, methanethiol and dimethyl sulfide were the only volatile compounds reaching higher levels in HPP-treated samples than in control samples (Martínez-Onandi et al., 2017). Albeit compositional characteristics and the length of refrigerated storage may interfere with the effect of HPP, it appears that the levels of some alcohols, aldehydes, ketones, alkanes and sulfur compounds tend to increase in HPP-treated samples. By inactivating the enzymes involved in the degradation of compounds or by hindering the access of those enzymes to their substrates, HPP might be responsible for the higher levels of some volatile compounds in HPP-treated ham samples. The opposite effect following HPP, either enzyme activation or a more favorable access of enzymes to their substrates, which would enhance the formation of certain volatile compounds, seems a less feasible explanation for the above mentioned increases.

In the present study, 23 volatile compounds were at lower levels in HPP-treated samples than in control samples at the end of refrigerated storage, the highest difference being recorded for 2-pentanol and 2-hexanol which were at 2.0-fold higher levels in control samples than in HPP-treated samples. In previous works on the effect of HPP on the volatile fraction of dry-cured ham, the levels of 23 volatile compounds (Rivas-Cañedo et al., 2009b) or 6 volatile compounds (Martínez-Onandi et al., 2016a) declined in HPP-treated Serrano ham samples analyzed shortly after treatment while in HPP-treated Serrano ham samples analyzed after 5 months of refrigerated storage the levels of 9 volatile compounds declined. The highest number of volatile compounds declining due to HPP was

recorded for Iberian ham samples analyzed shortly after treatment, in which the levels of 31 compounds decreased (Martínez-Onandi et al., 2017).

Lipid oxidation reactions in dry-cured ham can be promoted by HPP (Cheftel & Culioli, 1997; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Fuentes, Utrera, Estévez, Ventanas, & Ventanas, 2014b).). Slight differences in hexanal concentration were recorded among Iberian ham samples treated at 200 to 600 MPa but hexanal concentration increased considerably after treatment at 800 MPa (Andrés et al., 2004b). The levels of lipid-derived linear aldehydes and the perception of rancid odor were higher after HPP treatment of Iberian ham at 600 MPa for 6 min at 12 ºC (Fuentes et al., 2010). On the contrary, no increase of lipid oxidation derived compounds was observed in sliced Serrano ham after treatment at 400 MPa for 10 min at 12 ºC (Rivas-Cañedo et al., 2009b) or at 600 MPa for 6 min at 21 ºC (Martínez-Onandi et al., 2016a). Enhancement of lipid oxidation by HPP treatment would explain the higher levels found in the present study for one carboxylic acid (nonanoic acid), two alcohols (1-penten-3-ol, 2-butoxyethanol), one aldehyde (decanal), three ketones (2-butanone, 2-pentanone, 2-hexanone) and one ester (*p*-nitrophenyl hexanoate), compounds all derived from lipid oxidation reactions, and for three sulfur compounds (methanethiol, carbon disulfide, dimethyl trisulfide), which can also be generated through lipid oxidation reactions. At the beginning of the refrigerated storage period, the enhancement of lipid oxidation by HPP treatment of Iberian ham only affected nonanal, hexadecane, methanethiol and dimethyl trisulfide (Martinez-Onandi et al., 2017).

A PCA was carried out on the total levels of 7 selected groups of volatile compounds in HPP-treated and untreated Iberian ham samples. Component 1, including ketones, furanones and miscellaneous compounds, explained 43.6 % of the variance, component 2, including alkanes and furanes, 27.0 % of the variance, and component 3, including esters and acids, 18.6 % of the variance.

In spite of 89.2 % of the variance being jointly explained by the first three components, this PCA did not achieve the separation of HPP-treated samples from control samples (Fig. 1).

In a second PCA carried out on 12 selected individual volatile compounds, the first three components jointly explained 87.2 % of the variance. Component 1, including 2-methylpropanal, 2-methyl-2-butenal, 3-methylthiopropanal, 2-methylbutanal and dimethylsulfide, explained 42.5 % of the variance, component 2, including ethyl 2-hydroxypropanoate, ethyl acetate, ethyl hexanoate and ethyl propanoate, 27.8 % of the variance, and component 3, including 2-hexanone, 2-pentanone and *p*-nitrophenyl hexanoate, 16.9% of the variance. The separation of HPP-treated samples from control samples was improved with respect to the PCA on selected chemical groups, with a high proportion of HPP-treated samples located above the bisectrix of the first and third quadrants, although it was not fully satisfactory (Fig. 2).

3.3. Influence of prolonged refrigerated storage

The 116 volatile compounds found in Iberian ham after 5 months of storage at 4 ºC (Table 1) were present in all samples, independently of compositional characteristics or HPP treatment. This was a lower number than the 122 volatile compounds found in Iberian ham at the beginning of the refrigerated storage period (Martínez-Onandi et al., 2017). One hundred and nine compounds were present at the beginning and at the end of refrigerated storage while 13 compounds (1-hydroxy-2-butanone, 6-methyl-2-heptanone, ethyl butanoate, ethyl 3-methylbutanoate, ethyl heptanoate, nonane, hexadecane, 1-heptene, 2-octene, *m*-ethyltoluene, 2,4-dimethylphenol, phenylmethanol and pyrrole) were found only at the beginning of refrigerated storage, and 7 compounds (nonanoic acid, 2-ethyl-1-hexanol, butanal, decanal, acetophenone, ethyl ether and 2-methyl-1,3-thiazole) were detected only after 5 months at 4 ºC. In agreement with the lower number of volatile compounds, the sum of the levels of volatile compounds was on average 5.1 % lower at the end than at the

beginning of the refrigerated storage period in control hams and 1.3 % lower in HPP-treated hams, although those differences were not significant ($P > 0.05$). It must be noted that HPP treatment of Iberian ham caused a 5.1 % decrease in the sum of the levels of volatile compounds immediately after treatment (Martínez-Onandi et al., 2017) and refrigerated storage of untreated ham for 5 months also caused a 5.1 % decrease while HPP treatment followed by refrigerated storage accounted for a 6.3 % decrease.

The total levels of acids, alcohols, ketones, esters, sulfur compounds, furanes, furanones and pyrazines declined ($P < 0.05$) and those of benzene compounds increased ($P < 0.05$) during the refrigerated storage of control Iberian ham while in HPP-treated ham the total levels of acids, alcohols, ketones, esters, furanes, furanones and pyrazines declined ($P < 0.05$) and those of benzene compounds, sulfur compounds and miscellaneous compounds increased ($P < 0.05$). Regarding the levels of individual volatile compounds, 50 compounds either declined ($P < 0.05$) or disappeared during the refrigerated storage of Iberian ham while 25 compounds either increased ($P < 0.05$) or appeared during that period.

These results differ from those found for Serrano ham, in which 100 volatile compounds were detected at the beginning of the refrigerated storage period (Martínez-Onandi et al., 2016a) and 103 volatile compounds after 5 months at 4 ºC (Martínez-Onandi et al., 2016b), with 95 compounds present at the beginning and at the end of refrigerated storage. In the case of Serrano ham, the sum of the levels of volatile compounds was on average 32.7 % lower ($P < 0.05$) in control hams and 20.3 % lower ($P < 0.05$) in HPP-treated hams at the end than at the beginning of refrigerated storage. Total levels of alcohols, aldehydes, ketones, benzene compounds, sulfur compounds, furanones, terpenes and miscellaneous compounds declined ($P < 0.05$) during the refrigerated storage of control Serrano ham while in HPP-treated Serrano ham the total levels of alcohols, ketones, terpenes and miscellaneous compounds declined ($P < 0.05$) and those of furanes increased ($P < 0.05$). Thirty-six

individual volatile compounds either declined ($P < 0.05$) or disappeared during the refrigerated storage of Serrano ham while 13 compounds either increased ($P < 0.05$) or appeared.

The higher intramuscular fat content of Iberian ham might be responsible for its higher number of volatile compounds at the beginning of the refrigerated storage period (i.e. the end of ripening) in comparison with Serrano ham. Intramuscular fat content has been shown to influence lipid oxidation and sensory properties of HPP-treated Iberian ham (Fuentes, Estévez, Grèbol, Ventanas, & Ventanas, 2014a; Fuentes et al., 2014b). Factors other than compositional characteristics, such as the temperature during ripening, influence the formation and stability of volatile compounds in dry-cured ham (Andres et al., 2005; Zhang, Wang, Liu, Zhu, & Zhou, 2006). Afterwards, the evolvement and stability of volatile compounds during the refrigerated storage of Iberian and Serrano hams would be influenced not only by the intramuscular fat content but also by other compositional characteristics. In addition, the plastic packaging material may contribute to the volatile fraction of meat and meat products during refrigerated storage, in particular as a source of hydrocarbons, benzene compounds and aldehydes, as shown for HPP-treated and untreated fresh meats and dry-cured ham (Rivas-Cañedo, Fernández-García, & Nuñez, 2009a, b).

In control Iberian ham analyzed at the end of the refrigerated storage period, compounds presumably derived from lipid oxidation, compounds derived from Maillard reactions and compounds of microbial or unknown origin accounted for 75.1 %, 18.7 % and 6.2 %, respectively, of the volatile fraction. The percentages of these three groups of compounds were very close to those recorded for control Iberian ham at the beginning of refrigerated storage, time at which they accounted for 75.0 %, 18.1 % and 6.9 %, respectively, of the volatile fraction (Martínez-Onandi et al., 2017). As a whole, those data point to similar rates of the reactions involved in the formation and the degradation of the volatile compounds included in the three groups of compounds originating through different biochemical routes at the low temperature maintained during the

refrigerated storage of Iberian ham. In a previous study on the volatile fraction of ripened non-refrigerated Iberian ham the percentages of those three groups of volatile compounds were 81.6 %, 12.7 % and 5.7 %, respectively (Ramírez & Cava, 2007).

4. Conclusions

The compositional characteristics of Iberian dry-cured ham influenced the levels of 11 out of the 116 volatile compounds found after 5 months of storage at 4 ºC, with 5 compounds affected by intramuscular fat content and 7 compounds affected by salt concentration, salt-in-lean ratio or water activity. HPP treatment of Iberian ham had a significant effect on 34 volatile compounds, 11 of which were present at higher levels in HPP-treated samples and 23 in control samples. Refrigerated storage of Iberian ham had a significant effect on the levels of 75 volatile compounds, 50 of which disappeared or declined and 25 appeared or increased during the refrigerated storage period. Regarding their influence on the sum of the levels of all the volatile compounds found in Iberian ham, HPP caused a 5.1 % decrease immediately after treatment and refrigerated storage of untreated ham also caused a 5.1 % decrease while HPP treatment followed by refrigerated storage accounted for a 6.3 % decrease. Although HPP treatment and refrigerated storage are advantageous industrial and commercial procedures, their effect on the volatile fraction of Iberian ham must be taken into consideration. The most adequate HPP treatment conditions and the length of commercial shelf life should be established in order to maintain the unique odor and aroma characteristics of Iberian ham.

Acknowledgments

The authors wish to thanks E. Fulladosa, P. Gou and J. Arnau (IRTA, Monells, Spain) for the selection and processing of hams, the Spanish Ministry of Science and Innovation for project

RTA2010-00029-C04-04, which supported the experimental work, and INIA for funding the Ph. D. grant of N. Martínez-Onandi.

References

Andrés, A. I., Adamsen, C. E., Møller, J. K. S., Ruiz, J., & Skibsted, L. H. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research and Technology, 222*, 486-491.

Andrés, A. I., Cava, R., Martin, D., Ventanas, J., & Ruiz, J. (2005). Lipolysis in dry-cured ham: Influence of salt content and processing conditions. *Food Chemistry, 90*, 523-533.

Andrés, A. I., Cava, R., Ventanas, J., Muriel, E., & Ruiz, J. (2004a). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry, 84*, 375-381.

Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2007). Effect of salt content and processing conditions on volatile compounds formation throughout the ripening of Iberian ham. *European Food Research and Technology, 225*, 677-684.

Andrés, A. I., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2004b). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology, 219*, 205-210.

Armenteros, M., Toldrá, F., Aristoy, M. C., Ventanas, J., & Estévez, M. (2012). Effect of the partial replacement of sodium chloride by other salts on the formation of volatile compounds during ripening of dry-cured ham. *Journal of Agricultural and Food Chemistry, 60*, 7607-7615.

Carrapiso, A. I., & García, C. (2004). Iberian ham headspace: odorants of intermuscular fat and differences with lean. *Journal of the Science of Food and Agriculture, 84*, 2047-2051.

Carrapiso, A. I., Noseda, B., García, C., Reina, R., Sánchez del Pulgar, J., & Devlieghere, F. (2015). SIFT-MS analysis of Iberian hams from pigs reared under different conditions. *Meat Science, 104*, 8-13.

Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science and Emerging Technologies, 10*, 76-81.

Coutron-Gambotti, C., Gandemer, G., Rousset, S., Maestrini, O., & Casabianca, F. (1999). Reducing salt content of dry-cured ham: effect on lipid composition and sensory attributes. *Food Chemistry, 64*, 13-19.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat. *Meat Science, 46*, 211-236.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science, 85*, 506-514.

Fuentes, V., Estévez, M., Grèbol, N., Ventanas, J., & Ventanas, S. (2014a). Application of time-intensity method to assess the sensory properties of Iberian dry-cured ham: effect of fat content and high-pressure treatment. *European Food Research and Technology, 238*, 397-408.

Fuentes, V., Utrera, M., Estévez, M., Ventanas, J., & Ventanas, S. (2014b). Impact of high pressure treatment and intramuscular fat content on colour changes and protein and lipid oxidation in sliced and vacuum-packaged Iberian dry-cured ham. *Meat Science, 97*, 468-474.

García-Esteban, M., Ansorena, D., & Astiasarán, I. (2004). Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality. *Meat Science*, *67*, 57-63.

Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies, 5*, 451-457.

Hereu, A., Bover-Cid, S., Garriga, M., & Aymerich, T. (2012). High hydrostatic pressure and biopreservation of dry-cured ham to meet the Food Safety Objectives for *Listeria monocytogenes*. *International Journal of Food Microbiology, 154*, 107-112.

Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology, 110*, 8-18.

Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., & Picon, A. (2016a). Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. *Meat Science, 111*, 130-138.

Martínez-Onandi, N., Rivas-Cañedo, A., Picon, A., & Nuñez, M. (2016b). Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage. *Meat Science, 122*, 101-108.

Martínez-Onandi, N., Rivas-Cañedo, A., Ávila, M., Garde, S., Nuñez, M., & Picon, A. (2017). Influence of physicochemical characteristics and high pressure processing on the volatile fraction of Iberian dry-cured ham. *Meat Science*, 131, 40-47.

Morales P., Calzada J., Nuñez M. (2006) Effect of high pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. *Journal of Food Protection, 69*, 2539-2543.

Motilva, M. J., Toldrá, F., Nieto, P., & Flores, J. (1993). Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chemistry, 48*, 121-125.

Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: a review. *Grasas y Aceites, 63*, 432-454.

Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry, 55*, 1923-1931.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009a). Volatile compounds in fresh meats subjected to high pressure processing: Effect of the packaging material. *Meat Science, 81*, 321-328.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009b). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing. Effect of the packaging material. *Meat Science, 82*, 162-169.

Rodríguez, M., Nuñez, F., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1996). Gram-positive, catalase-positive cocci of dry-cured Iberian ham and their enterotoxigenic potential. *Applied and Environmental Microbiology, 62*, 1897-1902.

Santos-Garcés, E., Gou, P., Arnau, J., Fulladosa, E. (2010). Nondestructive analysis of a_w, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering, 101*, 187-192.

Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. *Trends in Food Science & Technology, 17*, 164-168.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science, 38*, 331-352.

Toldrá, F., Flores, M., & Sanz, Y. (1997). Dry-cured ham flavour: enzymatic generation and process influence. *Food Chemistry, 59*, 523-530.

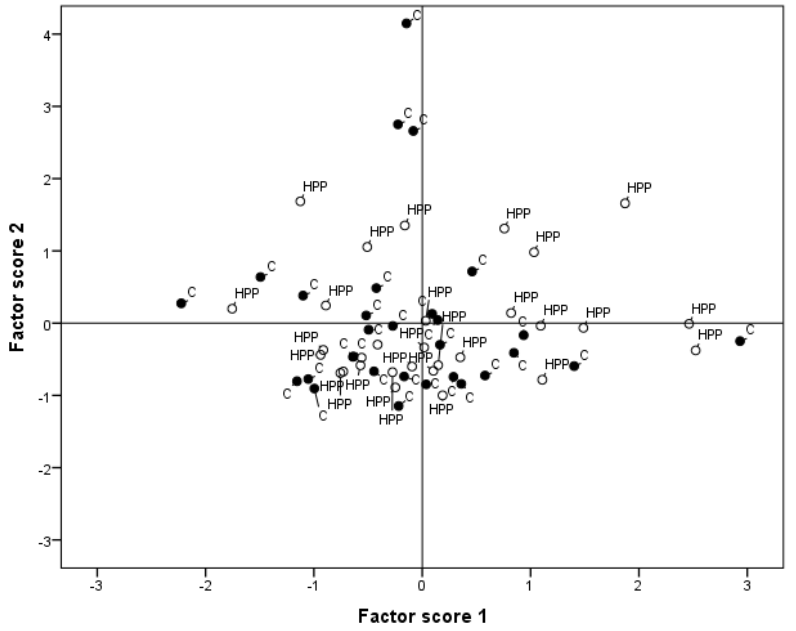
Ventanas, S., Estevez, M., Andrés, A.I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME-DED. *Meat Science, 79*, 172-180.

Wang, J., Jin, G., Zhang, W., Ahn, D. U., & Zhang, J. (2012). Effect of curing salt content on lipid oxidation and volatile flavour compounds of dry-cured turkey ham. *LWT-Food Science and Technology, 48*, 102-106.

Zhang, J., Wang, L., Liu, Y., Zhu, J., & Zhou, G. (2006). Changes in the volatile flavour components of Jinhua ham during the traditional aging process. *International Journal of Food Science and Technology, 41*, 1033-1039.

Zhang, J., Zhen, Z., Zhang, W., Zeng, T., & Zhou, G. (2009). Effect of intensifying high-temperature ripening on proteolysis, lipolysis and flavor of Jinhua ham. *Journal of the Science of Food and Agriculture, 89*, 834-842.

Zhou, G. H., & Zhao, G. M. (2007). Biochemical changes during processing of traditional Jinhua ham. *Meat Science, 77*, 114-120.



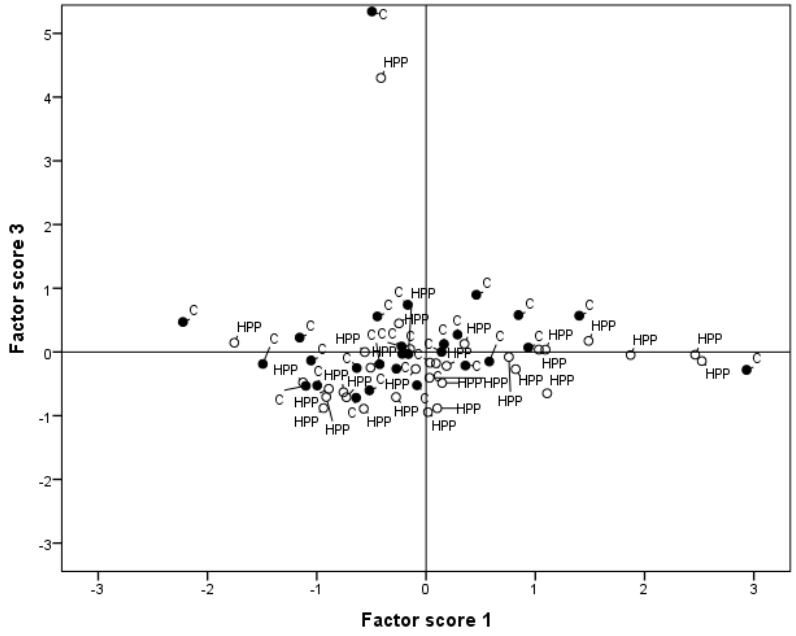


Fig. 1. Distribution of HPP-treated (HPP) and untreated control (C) samples of Iberian ham after refrigerated storage for 5 months at 4 ºC, according to the first three components (factor scores) of the PCA carried out on 7 selected groups of volatile compounds.

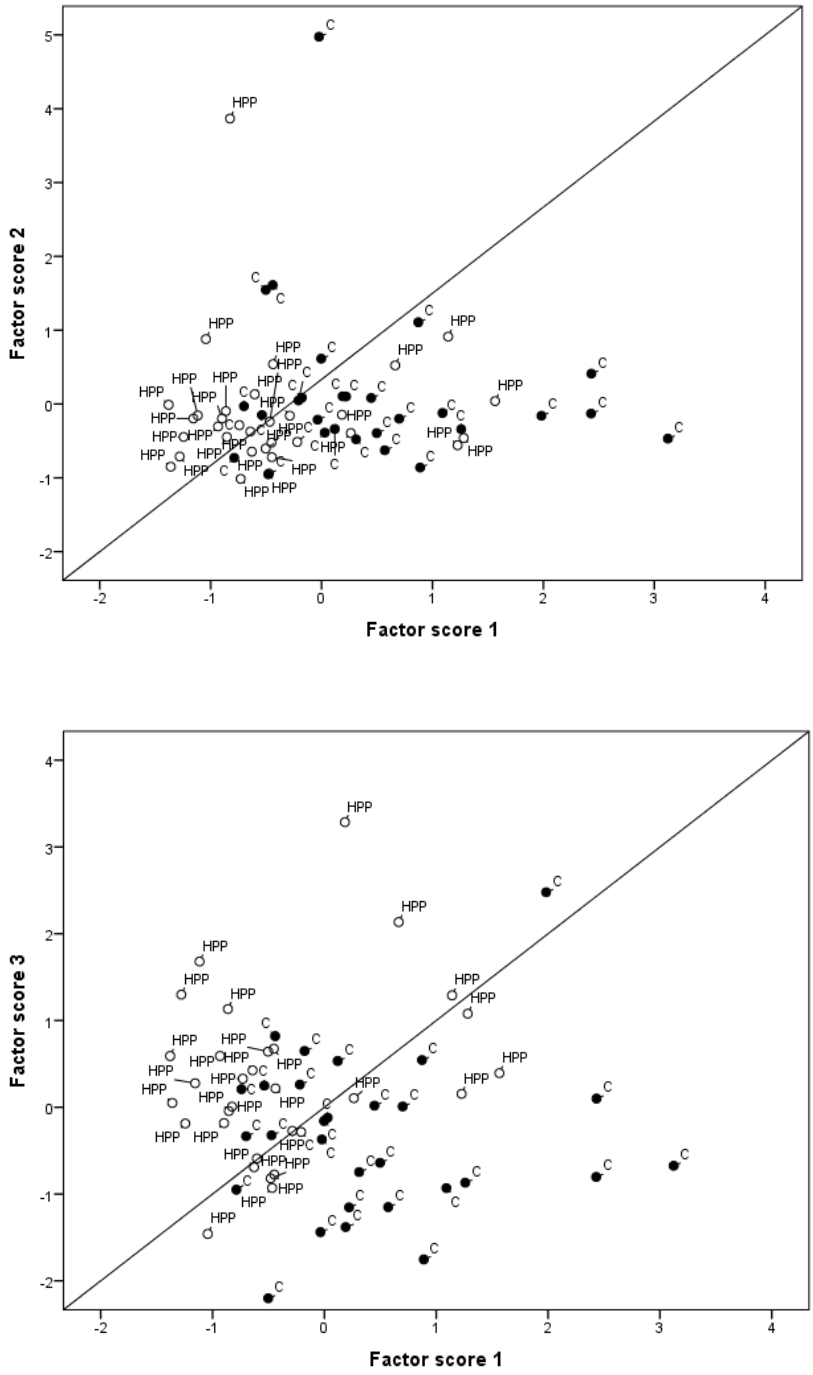


Fig. 2. Distribution of HPP-treated (HPP) and untreated control (C) samples of Iberian ham after refrigerated storage for 5 months at 4 ºC, according to the first three components (factor scores) of the PCA carried out on 12 selected individual volatile compounds.

Table 1. Volatile compounds detected in untreated and HPP-treated Iberian hams after refrigerated storage for 5 months at 4 ºC

Compound	LRI[a]	QI[b]	ID[c]
Acids			
Acetic acid	1469	43,45,60	ST, MS
Propanoic acid	1549	45,57,74	ST, MS
Butanoic acid	1641	60,73,88	ST, MS
Pentanoic acid	1682	43,60,87	ST, MS
Hexanoic acid	1855	41,60,87	ST, MS
Heptanoic acid	1968	43, 60, 73	ST, MS
Octanoic acid	2063	43,73,101,115	ST, MS
Nonanoic acid	2175	43,55,60,73,87	ST, MS
2-Methylpropanoic acid	1578	43,73,88	ST, MS
2-Methylbutanoic acid	1679	57,74	ST, MS
3-Methylbutanoic acid	1681	43,60	ST, MS
Alcohols			
Ethanol	941	45,46,43,41	ST, MS
1-Propanol	1059	59, 42, 60	ST, MS
1-Butanol	1165	56,41,43,42	ST, MS
1-Pentanol	1265	42,55,41,70	ST, MS
1-Hexanol	1364	56,55,69,41	ST, MS
1-Heptanol	1479	70,56,55,69	ST, MS
1-Octanol	1582	56,55,69,70	ST, MS
2-Propanol	934	45,43,41,59	ST, MS
2-Pentanol	1141	45,73	ST, MS
2-Hexanol	1236	45,43,69,57	ST, MS
2-Heptanol	1326	45,70,83,98	ST, MS
2-Methyl-1-propanol	1112	42,43,41,74	ST, MS
3-Methyl-1-butanol	1225	55,70,42,43	ST, MS
2-Ethyl-1-hexanol	1515	43,55,57,70	ST, MS
1-Penten-3-ol	1177	57,41,43,88	ST, MS
1-Octen-3-ol	1471	57,72,55	ST, MS
2-Methyl-3-buten-2-ol	1060	53,59,65,71	ST, MS
2-Butoxyethanol	1431	57,75,87,100,108	ST, MS
1-Methoxy-2-propanol	1148	45,59,75,90	ST, MS
Aldehydes			
Ethanal	704	41,42,43,44	ST, MS
Butanal	875	39,41,43,72	ST, MS
Pentanal	982	41,4457,58	ST, MS
Hexanal	1097	56,57,72	ST, MS
Heptanal	1199	70,44,43,55	ST, MS
Octanal	1299	56,57,84	ST, MS
Nonanal	1413	57,98,70,82	ST, MS
Decanal	1739	57,82,68,96	ST, MS

2-Methylpropanal	816	41,43,72	ST, MS
2-Methylbutanal	917	57,41,58,39	ST, MS
3-Methylbutanal	921	44,58,41,43	ST, MS
2-Methyl-2-butenal	1101	83,82	ST, MS
3-Methylthiopropanal	1479	47,48,76,104	ST, MS
Ketones			
2-Propanone	819	58,43,42,39	ST, MS
2-Butanone	907	43,72,57	ST, MS
2-Pentanone	985	43,41,86,57	ST, MS
2-Hexanone	1089	58,85,100	ST, MS
2-Heptanone	1198	43,58,71,85	ST, MS
2-Octanone	1300	43,58,71,59	ST, MS
2-Nonanone	1402	58,71	ST, MS
3-Heptanone	1164	57,85,114	ST, MS
3-Octanone	1262	72,99,128	ST, MS
2,3-Pentadione	1069	43,57,100	ST, MS
2,3-Octadione	1326	43,71,99	MS
1-(2-Furanyl)-ethanone	1060	67,95,110	MS
1-Hydroxy-2-propanone	1314	43,74	ST, MS
3-Hydroxy-2-butanone	1303	43,45,73,88	ST, MS
3-Ethylcyclopentanone	1348	70,83,97,112	MS
4-Hydroxy-4-methyl-2-pentanone	1379	43,58	ST, MS
Esters			
Ethyl acetate	894	43,45,61,70	ST, MS
Ethyl propanoate	957	57,74,75,102	ST, MS
Ethyl hexanoate	1246	55,60,70,88,99,115	ST, MS
Ethyl octanoate	1453	88,127	ST, MS
Ethyl decanoate	1661	88, 101, 157	ST, MS
Ethyl 2-hydroxypropanoate	1352	45,75	ST, MS
Ethyl 2-methylbutanoate	1070	57,74,85,102,115	ST, MS
Alkanes			
Pentane	500	41,42,57,72	ST, MS
Hexane	600	57,41,56,42	ST, MS
Heptane	700	43,57,71,41	ST, MS
Octane	800	43,57,85,71	ST, MS
Decane	1000	43,57,71,85,99	ST, MS
Cyclohexane	724	56,84	ST, MS
2,2,4,6,6-Pentamethylheptane	941	57,56,71,85	ST, MS
Branched chain alkane I	1005	71,85,111,127	MS
Branched chain alkane II	1032	57,71,113	MS
Benzene compounds			
Benzene	939	51.52,77.78	ST, MS
Methylbenzene	1055	91,92,65,93	ST, MS
1,2,3-Trimethylbenzene	1293	105,120	ST, MS
Ethylbenzene	1131	91,106,65,51	ST, MS
1-Methylethylbenzene	1183	79,105,120	ST, MS
o-Ethyltoluene	1231	105,120	ST. MS
Styrene	1272	104, 103, 78, 77	ST, MS
Benzaldehyde	1556	77,105,106,51	ST, MS

Benzonitrile	1644	50,76,103	ST, MS
Phenol	2037	66,94	ST, MS
4-Methylphenol	2093	77,90,107	ST, MS
4-Ethylphenol	1226	77,107,122	ST, MS
4-Pentylphenol	1497	107,164	ST, MS
o-Xylene	1199	91,106,105,77	ST, MS
m-Xylene	1157	91,106,105,77	ST, MS
p-Xylene	1150	91,106	ST, MS
Acetophenone	1687	77,105,120	ST, MS
2-Phenylethanol	1970	65,91,92,122	ST, MS
Phenylethanal	1698	43,60	ST, MS
1-Phenylpropane	1218	91,92,120	ST, MS
Naphthalene	1800	51,64,128	ST, MS
Sulfur compounds			
Methanethiol	678	47,48,45,46	ST, MS
Carbon disulfide	729	76,78,77,64	ST, MS
Dimethyl sulfide	747	35,47,61,62	ST, MS
Dimethyl disulfide	1086	94,79,46	ST, MS
Dimethyl trisulfide	1402	126,45,47,79	ST, MS
Furanes			
2-Methylfuran	871	51,81,82	ST, MS
2-Ethylfuran	952	53,81,96,51,82	ST, MS
2-Buthylfuran	1141	53,81,124	ST, MS
2-Pentylfuran	1240	81,82,95,53,138	ST, MS
Furanones			
Dihydro-2(3H)-furanone	1652	42,56,86	ST, MS
5-Methyldihydro-2(3H)-furanone	1673	56,86	ST, MS
5-Ethyldihydro-2(3H)-furanone	1756	56,70,85	ST, MS
5-Butyldihydro-2(3H)-furanone	1865	56,85	MS
5-Pentyldihydro-2(3H)-furanone	1988	85,124	MS
Pyrazines			
Methylpyrazine	1285	40,53,67,94	ST, MS
2,6-Dimethylpyrazine	1346	53,67,81,93	ST, MS
2,3,5-Trimethylpyrazine	1437	122,42,81,39	ST, MS
Miscellaneous compounds			
Ethyl ether	620	45,59,74	ST, MS
2-Methyl-1,3-thiazole	1255	58,99	ST, MS
p-Nitrophenyl hexanoate	1696	43,55,71,99	ST, MS

[a] LRI: Linear retention indexes, calculated in relation to the retention time of n-alkane (C5-C20) series.
[b] QI: Ions used for quantification.
[c] ID: Peak identification: ST, comparison of spectra and retention time with commercial standards; MS, tentatively identified by spectra comparison using the Wiley Library.

Table 2. Levels[1] of the 5 volatile compounds significantly influenced by intramuscular fat content in untreated or HPP-treated Iberian hams after refrigerated storage for 5 months at 4 ºC

Compound	Untreated ham				HPP-treated ham			
	Low fat (n=10) < 11.32 %	Medium fat (n=11) 11.32-13.44 %	High fat (n=9) > 13.44 %	P^2	Low fat (n=10) < 11.32 %	Medium fat (n=11) 11.32-13.44 %	High fat (n=9) > 13.44 %	P^2
2-Pentanol	157.97±64.25^{b}	97.31±23.87^{a}	97.38±28.20^{a}	*	71.64±26.45^{a}	51.80±16.09^{a}	53.52±21.74^{a}	ns
2-Hexanol	6.42±3.35^{b}	4.08±1.07^{ab}	3.50±0.97^{a}	*	2.76±0.98^{a}	2.16±0.62^{a}	2.12±0.66^{a}	ns
Ethanal	8.72±1.61^{ab}	7.74±2.21^{a}	10.16±2.15^{b}	*	7.42±2.28^{a}	6.59±1.90^{a}	8.60±2.00^{a}	ns
Pentane	114.85±52.80^{a}	103.49±30.39^{a}	141.77±42.94^{a}	ns	105.76±32.29^{a}	121.26±32.57^{ab}	160.68±41.55^{b}	*
2-Phenylethanol	128.11±38.10^{b}	86.55±25.61^{a}	118.30±27.18^{ab}	*	129.19±42.21^{a}	93.54±24.14^{a}	126.14±34.47^{a}	ns

[1]Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. Means bearing different superscript differ significantly.

[2]Statistical significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3. Levels[1] of the 5 volatile compounds significantly influenced by salt concentration in untreated or HPP-treated Iberian hams after refrigerated storage for 5 months at 4 ºC

Compound	Untreated ham				HPP-treated ham			
	Low salt (n=12) < 4.23 %	Medium salt (n=7) 4.23-4.72 %	High salt (n=11) > 4.72 %	P^2	Low salt (n=12) < 4.23 %	Medium salt (n=7) 4.23-4.72 %	High salt (n=11) > 4.72 %	P^2
Acetic acid	589.86±92.50^{ab}	627.99±169.85^{b}	506.36±42.06^{a}	*	560.08±70.87^{ab}	603.22±181.05^{b}	468.30±68.62^{a}	*
Propanoic acid	22.35±3.22^{ab}	33.06±12.68^{b}	19.64±1.97^{a}	*	21.53±2.81^{ab}	29.30±14.81^{b}	18.45±3.12^{a}	*
Nonanoic acid	2.51±0.65^{ab}	2.78±0.82^{b}	1.99±0.37^{a}	*	3.08±0.59^{a}	3.22±0.84^{a}	2.64±0.73^{a}	ns
3-Methyl-1-butanol	194.60±44.36^{a}	175.77±67.15^{a}	146.93±28.41^{a}	ns	200.66±41.61^{b}	176.04±53.13^{ab}	145.06±30.97^{a}	*
Phenylethanal	60.83±8.33^{ab}	67.31±26.49^{b}	50.52±9.30^{a}	*	52.29±11.95^{a}	54.24±25.69^{a}	46.35±11.53^{a}	ns

[1]Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. Means bearing different superscript differ significantly.

[2]Statistical significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4. Levels[1] of the 4 volatile compounds significantly influenced by salt-in-lean (S/L) ratio in untreated or HPP-treated Iberian hams after refrigerated storage for 5 months at 4 ºC

Compound	Untreated ham				HPP-treated ham			
	Low S/L (n=10) < 0.048	Medium S/L (n=9) 0.048-0.054	High S/L (n=11) > 0.054	P^2	Low S/L (n=10) < 0.048	Medium S/L (n=9) 0.048-0.054	High S/L (n=11) > 0.054	P^2
Acetic acid	561.91±98.54^{ab}	637.11±135.80^{b}	506.33±46.07^{a}	*	564.38±93.34^{ab}	577.21±122.56^{b}	465.56±74.63^{a}	*
3-Methyl-1-butanol	197.63±52.78^{a}	170.66±44.51^{a}	149.15±30.25^{a}	ns	205.23±61.41^{b}	171.27±35.70^{ab}	146.46±33.60^{a}	*
Ethanal	10.36±2.22^{b}	8.02±2.19^{a}	7.99±1.55^{a}	*	8.30±2.39^{a}	6.89±1.85^{a}	7.18±2.07^{a}	ns
Cyclohexane	393.96±111.23^{a}	427.63±109.58^{a}	387.46±42.62^{a}	ns	485.61±112.12^{b}	397.96±70.87^{ab}	371.03±68.71^{a}	*

[1]Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. Means bearing different superscript differ significantly.

[2]Statistical significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5. Levels[1] of the 4 volatile compounds significantly influenced by a_w in untreated or HPP-treated Iberian hams after refrigerated storage for 5 months at 4 ºC

Compound	Untreated ham				HPP-treated ham			
	Low a_w (n=11) < 0.871	Medium a_w (n=9) 0.871-0.879	High a_w (n=10) > 0.879	P^2	Low a_w (n=11) < 0.871	Medium a_w (n=9) 0.871-0.879	High a_w (n=10) > 0.879	P^2
Acetic acid	506.33±46.07^a	637.11±135.80^b	561.91±98.54^{ab}	*	465.56±74.63^a	577.21±122.56^b	564.38±93.34^{ab}	*
3-Methyl-1-butanol	149.15±30.25^a	170.66±44.51^a	197.63±52.78^a	ns	146.46±33.60^a	171.27±35.70^{ab}	205.23±61.41^b	*
Ethanal	7.99±1.55^a	8.02±2.19^a	10.36±2.22^b	*	7.18±2.07^a	6.89±1.85^a	8.30±2.39^a	ns
Cyclohexane	387.46±42.62^a	427.63±109.58^a	393.96±111.23^a	ns	371.03±68.71^a	397.96±70.87^{ab}	485.61±112.12^b	*

[1]Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}. Means bearing different superscript differ significantly.

[2]Statistical significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 6. Levels[1] of the 34 volatile compounds of Iberian ham significantly influenced by high pressure processing (HPP) after refrigerated storage for 5 months at 4 ºC

Compound	Untreated ham (n=30)	HPP-treated ham (n=30)	P^2
Acetic acid	564.09±36.89^b	532.00±35.94^a	*
Propanoic acid	23.41±3.71^b	21.85±2.89^a	*
Hexanoic acid	187.86±17.90^b	175.42±16.12^a	*
Nonanoic acid	2.36±0.22^a	2.93±0.25^b	***
2-Pentanol	117.55±18.62^b	58.93±8.72^a	***
2-Hexanol	4.68±0.81^b	2.35±0.30^a	***
2-Heptanol	39.95±6.17^b	22.54±4.17^a	***
1-Penten-3-ol	53.63±4.21^a	56.96±4.02^b	*
2-Butoxyethanol	40.64±6.17^a	44.89±7.80^b	*
Ethanal	8.79±0.74^b	7.47±0.72^a	***
Butanal	6.37±0.51^b	5.53±0.44^a	***
Pentanal	60.44±6.82^b	53.62±5.66^a	**
Hexanal	610.54±55.38^b	531.01±40.45^a	**
Heptanal	65.59±7.73^b	57.46±5.09^a	*
Decanal	5.34±0.89^a	10.19±1.62^b	**
2-Methylpropanal	96.89±7.92^b	78.82±7.43^a	***
2-Methylbutanal	401.95±3.11^b	356.00±30.06^a	***
3-Methylbutanal	527.11±38.00^b	465.64±33.94^a	***
2-Methyl-2-butenal	3.89±0.35^b	3.48±0.35^a	***
3-Methylthiopropanal	23.55±2.03^b	20.48±2.29^a	***
2-Butanone	210.63±18.24^a	226.66±18.15^b	**
2-Pentanone	94.85±13.82^a	121.79±15.55^b	***
2-Hexanone	74.97±8.57^a	79.49±8.09^b	*
Ethyl acetate	35.86±6.58^b	30.75±5.33^a	***
Ethyl propanoate	3.67±0.87^b	2.99±0.67^a	***

Ethyl hexanoate	18.89±3.11[b]	16.17±2.13[a]	**
Ethyl-2-hydroxypropanoate	8.20±1.82[b]	7.23±1.56[a]	**
Phenyl-ethanal	58.00±4.83[b]	50.30±4.93[a]	***
Methanethiol	9.14±1.19[a]	15.57±1.54[b]	***
Carbon disulfide	39.86±6.24[a]	45.51±6.27[b]	*
Dimethyl sulfide	5.69±0.96[b]	3.23±0.37[a]	***
Dimethyl trisulfide	46.03±7.97[a]	203.86±31.89[b]	***
2(3H)-5-Pentyldihydrofuranone	11.92±1.16[b]	11.24±1.01[a]	*
p-Nitrophenyl hexanoate	21.47±2.60[a]	27.12±3.22[b]	***

[1] Levels (mean ± SE) are the sum of the abundances of characteristic ions, multiplied by 10^{-5}.

[2] Statistical significance in the analysis of variance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

7. Microbiota of Iberian dry-cured ham as influenced by chemical composition, high pressure processing and prolonged refrigerated storage

Manuscrito en preparación

Microbiota of Iberian dry-cured ham as influenced by chemical composition, high pressure processing and prolonged refrigerated storage

N. Martínez-Onandi, C. Sánchez, M. Nuñez, A. Picon

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña km 7, 28040, Madrid, Spain

Abstract

The effect of high pressure processing (HPP) on the microbiota of ripened Iberian ham of different chemical composition was investigated before and after a 5-month refrigeration period. At the beginning of the refrigeration period, the only significant effects of chemical composition were those of water activity on psychrotrophs and *Micrococcaceae* in untreated hams, and of the salt-in-lean ratio on lactic acid bacteria in HPP-treated hams. At the end of the refrigeration period, the only significant effect was that of intramuscular fat content on moulds and yeasts in HPP-treated samples. All investigated microbial groups were significantly affected by HPP, with reductions ranging from 1.7 to 2.0 log cycles immediately after treatment. A significant recovery of all microbial groups took place in HPP-treated hams during the refrigeration period, with increases ranging from 0.5 to 1.1 log cycles. In spite of this recovery, levels in HPP-treated hams were still significantly lower than in untreated hams. A percentage of 96.0 % of Iberian ham bacterial isolates belonged to the *Staphylococcus* genus, with *S. equorum* as the most abundant species. Isolates belonging to the genera *Tetragenococcus* and *Carnobacterium* isolates, which had not been previously reported in dry-cured ham, were also detected. Most yeast isolates were identified as *Debaryomyces hansenii*.

Keywords: Iberian ham, microbiota, water activity, salt, intramuscular fat, high pressure processing.

1. Introduction

Iberian ham is a dry-cured meat product highly appreciated by consumers due to its high sensory quality, partly due to the characteristics of the raw material (acorn-fed autochthonous pig breed) and the length of the manufacturing process (Ruiz et al., 2002). The manufacturing process of Iberian ham basically consists in four stages: a conditioning step, a salting step, a post-salting step and a ripening period, which may last up to 24 months (Toldrá & Flores, 1998). During the salting step, a microbial community mostly consisting of microorganisms from the salt colonises the ham surface (Cornejo et al., 1992). During the post-salting step, salt diffusion to the inner zones, water loss, and low temperatures result in a gradual predominance of the salt-tolerant microbiota (Blesa et al., 2008).

Micrococacceae, moulds and yeasts have been reported as the dominant microorganisms in dry-cured ham (Huerta et al., 1988; Núñez et al., 1996a, b). *Micrococcaceae* constitutes the predominant microbial group in the salt used in the manufacture of dry-cured ham from white pig breed (Cordero & Zumalacárregui, 2000). In Iberian ham, most of the *Micrococcaceae* isolates belonged to the *Staphylococcus* genus, being *S. xylosus* followed by *S. equorum* the predominant species (Rodríguez et al., 1994). The presence of *S. equorum* in ham may have been underestimated because of its confusion with *S. xylosus* when isolates were identified by phenotypical and biochemical methods (Blaiotta et al., 2004; Landeta et al., 2011). Staphylococci play important roles in colour formation, through their nitrate reductase activity, inhibition of rancidity, through their antioxidant activity, and flavour development, via the catabolism of branched-chain amino acids and pyruvate (Vermassen et al., 2016).

Moulds are considered beneficial in the ripening of dry-cured ham due to their positive effects on flavour and external appearance (Núñez et al., 1996a). However,

production of mycotoxins should be considered a health hazard. Species of the *Penicillium*, *Aspergillus* and *Eurotium* genera were detected in Iberian ham as the predominant moulds. Members of the *Aureobasidium*, *Cladosporium*, *Curvularia* and *Syncephalastrum* genera were also detected (Núñez et al., 1996a). Among yeasts, *Debaryomyces hansenii* and *Candida zelanoides* were the predominant species in Iberian ham (Núñez et al., 1996b**).** *D. hansenii* showed an intense proteolytic activity against pork myofibrillar proteins (Rodríguez et al., 1998; Martín et al., 2001).

The meat industry tries to satisfy consumers demand for high quality, tasty, healthy, natural and safe meat products with an extended shelf life. In the specific case of dry-cured ham, consumers demand low salt and fat contents. Nevertheless, salt is an essential ingredient in the manufacturing process of dry-cured ham since it prevents microbial growth, reduces water activity (a_w), controls enzyme action, facilitates the solubilisation of some proteins and confers a typical salty taste to ham that enhances its flavour (Andrés et al., 2004). Salt reduction may increase microbiological risks and bring about technological problems (Armenteros et al., 2009). It has been shown that low salt Serrano ham had higher levels of volatile compounds coming from Strecker reactions and microbial metabolism than medium or high salt hams (Martínez-Onandi et al., 2016).

Although entire dry-cured ham is considered a shelf-stable product, the deboning, slicing and packaging operations characteristic of ready-to-eat products may increase the risk of microbial cross-contamination by spoilage and pathogenic microorganisms, compromising shelf life and safety. High pressure processing (HPP), a non-thermal preservation technology, is being widely used in the meat industry for controlling food spoilage and improving food safety, while retaining the characteristics of fresh, minimally processed foods. Microbial inactivation by HPP depends on treatment conditions (pressure level, holding time and treatment temperature), factors related to the

microorganism (species, strain, growth phase, etc), and food composition (pH, a_w, etc) (Rendueles et al., 2011). HPP of Serrano ham resulted in significant reductions of total bacterial levels after treatment, from 1.5 to 2.5 log cycles (de Alba et al., 2015; Garriga et al., 2004; Martínez-Onandi et al., 2017a). Chemical composition significantly affected counts of aerobic mesophiles, psychrotrophs, moulds and yeasts in Serrano ham (Martínez-Onandi et al., 2017a). However, to our knowledge, there is no available information on the effect of chemical composition on the microbiota of Iberian ham or on how chemical composition would modulate the effect of HPP on microorganisms.

The objective of the present study was to investigate the effect of ham chemical composition, HPP treatment and refrigerated storage for 5 months on the microbiota of ripened dry-cured Iberian ham.

2. Materials and Methods

2.1. Selection of Iberian hams

Two groups each consisting of 30 Iberian hams were selected from a batch of 120 hams produced at a processing plant in Extremadura (Spain) from 50 % Iberian x Duroc animals. Selection of hams was performed at the Institute of Food and Agricultural Research and Technology (IRTA, Monells, Spain) as previously described (Martínez-Onandi et al., 2017b). The first group of hams was analysed 3 days after HPP treatment and the second group after a 5-month refrigeration period, simulating commercial storage conditions.

2.2. Sampling and high pressure processing

Two slices (approximately 150 g) from the cushion (mainly composed of the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* muscles) were obtained from each ham and individually vacuum-packaged. HPP treatment at 600 MPa for 6 min at 21 ºC

(pressure build up time, 2.5 min; pressure release time < 2 s) in a 120 L capacity Wave 6000 equipment (Hiperbaric, Burgos, Spain) at IRTA was applied to one of the two slices whereas the other one served as untreated control. Ham slices were maintained at 4 ºC until microbiological analysis, which was carried out after 3 days or 5 months of storage at 4 ºC. After microbiological analysis, samples for chemical determinations were maintained at -35 ºC.

2.3. Physicochemical determinations

Representative ham homogenates were obtained with a mechanical grinder (IKA Labortechnik, Staufen, Germany). Chloride content was determined by the Volhard method (AOAC, 2000) and intramuscular fat (IMF) content by the Folch method (Folch et al., 1957). Water activity (a_w) was measured using an AquaLab Series 3 equipment (Decagon, Devices, Inc., Pullman, WA, USA). Analyses were performed in triplicate.

2.4. Microbiological analysis

Representative ham samples (10 g) were aseptically taken and homogenized with 90 mL of a sterile saline peptone solution (Maximum recovery diluent, Biolife, Milano, Italy) in a Colworth Stomacher 400 (A. J. Seward Ltd., London, UK) for 3 minutes. Serial dilutions were prepared and plated in duplicate onto appropriate culture media. Aerobic mesophilic bacteria, psychrotrophs, *Enterobacteriaceae*, lactic acid bacteria, enterococci, *Micrococcaceae*, coagulase-positive staphylococci, and moulds and yeasts were respectively enumerated on Plate Count Agar (PCA, Biolife), PCA, Violet Red Bile Glucose Agar (VRBG, Biolife), MRS Agar (Biolife), Kanamycin Aesculin Azide Agar (KAA, Oxoid, Basingstoke, Hampshire, UK), Mannitol Salt Agar (MSA, Oxoid), Baird-Parker agar with rabbit plasma fibrinogen (RPF) Supplement II (BP+RPF, Biolife), and Sabouraud Dextrose Agar (SDA, Oxoid) as previously described (Martínez-Onandi et al., 2017a). Microbial counts were expressed in log cfu per gram of ham. The presence of *L.*

monocytogenes and *Salmonella* spp. in 25 g of ham was investigated by pre-enrichment, enrichment and selective isolation on specific media as previously described (Martínez-Onandi et al., 2017a).

2.5. Isolation of microorganisms and DNA extraction

Two hundred twenty seven representative bacterial isolates from different solid culture media (PCA, MSA, BP+RPF and MRS) were purified by streaking them three times on the same medium. Isolates thus obtained were stored at - 40 ºC in liquid cultures supplemented with glycerol (20 % w/v).

Total genomic DNA from pure cultures in the late exponential growth phase was isolated with the GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, Tres Cantos, Spain), following the manufacturer's recomendation.

Thirty yeast isolates were also purified and stored in liquid cultures as previously described. Total genomic DNA from pure yeast cultures was isolated with the Yeast DNA Extraction kit (Thermo Fisher Scientific, Madrid, Spain).

2.6. Molecular identification of isolates

An approximately 800 basepair (bp) region of the *16S rDNA* gene was amplified using the universal primers W01 and 800R. Polymerase chain reactions (PCR) were performed as previously described (Campos et al., 2011). Amplified PCR products from 96 isolates were purified using the GenElute PCR clean-up kit (Sigma-Aldrich) and sequenced at the Genomic Unit (Complutense University CAI, Madrid, Spain). The obtained sequences (forward and reverse) were compared to those deposited in databases BLAST (http://blast.ncbi.nlm.nih.gov/) and RDP (http://rdp.cme.mus.edu). With the obtained sequences, a partial amplified rDNA restriction analysis (partial ARDRA) was developed to identify the 131 remaining isolates. A first digestion with restriction endonuclease *Kpn*I (Thermo Fisher Scientific, Madrid, Spain) classified them in two

groups (digestible or non-digestible with *Kpn*I). *Kpn*I-digestible amplicons were subjected to the action of endonucleases *Mbo*I and *Ava*II, and were classified in three different groups. Amplicons with no *Kpn*I site and those digested with *Ava*II were subjected to the action of endonuclease *Taq*I. Restriction fragment patterns were analyzed in 2% (wt/vol) agarose in 0.5× Tris-borate-EDTA buffer using a 100-bp DNA ladder (Thermo Fisher Scientific) as molecular size marker.

For yeast isolates, the region spanning the internal transcribed spacers (ITS1 and 2) and the 5.8S rDNA gene was amplified using primers ITS1 and ITS4 as described (Guillamón et al., 1998). Purification, sequencing and comparison of the obtained sequences were performed as above described.

2.7. Statistical analysis

Microbial counts were analysed using the SPSS 19.0 statistical package (S.P.S.S. Inc., Chicago, IL, USA). Hams were divided in three groups (low, medium and high values) for each compositional parameter. The mean ± 0.5 standard deviation (SD) criterion was used to establish these three groups (Martínez-Onandi et al., 2016). One-way analysis of variance (ANOVA) was carried out with each of the compositional parameters (a_w, salt concentration, salt-in-lean ratio, intramuscular fat content) as main effect and ham as random effect or with HPP treatment as main effect and ham as fixed effect. Means were compared by Tukey's test, with the significance assigned at $P < 0.05$.

3. Results

3.1. Effect of ham chemical composition

In the first group of Iberian hams, analysed at the beginning of the 5-month refrigeration period, a_w ranged from 0.845 to 0.912, with a mean value of 0.876 (SD, 0.015). Hams were grouped into 6 low a_w (< 0.868) hams, 17 medium a_w (0.868-0.883)

hams and 7 high a_w (> 0.883) hams. Values of a_w significantly influenced counts of psychrotrophs and *Micrococcaceae* in untreated hams, with the highest levels of these microbial groups in high a_w hams, and had no significant effect on any of the microbial groups in HPP-treated hams (Table 1).

NaCl concentration of hams ranged from 3.02 % to 5.62 %, with a mean value of 4.27 % (SD, 0.67 %). Hams were grouped into 11 hams of low (< 3.93 %), 10 of medium (3.93-4.60 %) and 9 of high (> 4.60 %) salt content. Salt concentration did not significantly influence the counts of any microbial group in either untreated or HPP-treated hams (Table 1). Salt-in-lean (S/L) ratio of hams ranged from 0.036 to 0.063, with a mean value of 0.049 (SD, 0.005). Hams were grouped into 12 hams of low (< 0.045), 9 of medium (0.045-0.052) and 9 of high (> 0.052) S/L ratio. The S/L ratio did not affect the counts of any microbial group in untreated hams but it influenced the counts of lactic acid bacteria in HPP-treated hams, with the highest levels of this microbial group in hams of low S/L ratio (Table 1).

Intramuscular fat content of hams ranged from 4.63 % to 18.59 %, with a mean value of 11.96 % (SD, 3.56 %). Hams were grouped into 9 hams of low (< 10.18 %), 10 of medium (10.18-13.75 %) and 11 of high (> 13.75 %) IMF content. The IMF content did not significantly influence the counts of any microbial group in either untreated or HPP-treated hams (Table 1).

In the second group of Iberian hams, analysed after 5 months at 4 ºC, a_w ranged from 0.859 to 0.890, with a mean value of 0.875 (SD, 0.008). Hams were grouped into 11 low a_w (< 0.871), 9 medium a_w (0.871-0.879) and 10 high a_w (> 0.879) hams. Values of a_w did not significantly influence the counts of any microbial group in either untreated or HPP-treated hams (Table 2).

Salt concentration ranged from 3.51 % to 5.35 %, with a mean value of 4.48 % (SD, 0.50 %). Hams were grouped into 12 low salt (< 4.23 %), 7 medium salt (4.23-4.72 %) and 11 high salt (> 4.72 %) hams. Salt concentration did not significantly influence the levels of any microbial group in either untreated or HPP-treated hams (Table 2). The S/L ratio of hams ranged from 0.042 to 0.061, with a mean value of 0.051 (SD, 0.005). Hams were grouped into 10 low S/L (< 0.048), 9 medium S/L (0.048-0.054) and 11 high S/L (> 0.054) hams. The S/L ratio did not significantly influence the levels of any microbial group in either untreated or HPP-treated hams (Table 2).

Intramuscular fat content ranged from 8.87 % to 16.62%, with a mean value of 12.38 % (SD, 2.12 %). Hams were grouped into 10 low fat (< 11.32 %), 11 medium fat (11.32-13.44 %) and 9 high fat (> 13.44 %) hams. The IMF content did not affect the counts of any microbial group in untreated hams, although it significantly influenced the counts of moulds and yeasts in HPP-treated hams, with the highest levels of this microbial group in low and medium fat content hams (Table 2).

3.2. Effect of high pressure processing

Counts of the different microbial groups in untreated and HPP-treated hams, at the beginning and the end of the 5-month refrigerated storage period, are shown in Table 3. At both times, all the analysed microbial groups were significantly ($P < 0.05$) affected by the HPP treatment. Reductions of 1.78, 2.00, 2.04, 1.74 and 1.87 log cfu g^{-1} were recorded immediately after HPP for aerobic mesophiles, psychrotrophs, lactic acid bacteria, *Micrococcaceae*, and moulds and yeasts, respectively. Neither *L. monocytogenes* nor *Salmonella* spp. were detected after enrichment of untreated or HPP-treated Iberian ham 25 g samples.

At the end of the 5-month refrigeration period, counts of aerobic mesophiles, psychrotrophs, lactic acid bacteria, *Micrococcaceae* and moulds and yeasts were

respectively 0.53, 1.02, 1.34, 0.93 and 0.81 log cfu g^{-1} lower in HPP-treated ham samples than in untreated samples. *L. monocytogenes* and *Salmonella* spp. were not detected after refrigerated storage in any of the untreated or HPP-treated Iberian ham samples.

3.3. Effect of prolonged refrigerated storage

During the 5-month refrigeration period, levels of aerobic mesophiles and moulds and yeasts significantly decreased, those of lactic acid bacteria significantly increased and those of psychrotrophs and *Micrococcaceae* were not altered in untreated Iberian ham. In contrast, significant increases, ranging from 0.53 to 1.09 log cfu g^{-1}, in the levels of all microbial groups were observed in HPP-treated Iberian ham. In spite of those increases, the levels of all microbial groups remained significantly lower than those of untreated Iberian ham at the end of the refrigeration period. Since no significant differences in chemical composition between hams at the beginning and the end of the refrigerated storage were found, the observed differences were attributed to the effect of the prolonged refrigerated storage.

3.4. Microbial diversity

A fragment of approximately 800 bp of the *16S rDNA* gene was amplified by PCR using DNA from 113 bacterial isolates from untreated and HPP-treated hams at the beginning of the refrigeration period and from 114 bacterial isolates from hams at the end of the refrigeration period as template. Sequencing of 96 amplicons identified them as follows: 90 of them belonged to the genus *Staphylococcus*, 3 to *Tetragenococcus*, 2 to *Enterococcus* and 1 to *Carnobacterium*. Among *Staphylococcus*, the following species were found: *S. equorum* (44 isolates), *S. epidermidis* (21 isolates), *S. pasteuri*/*warneri* (22 isolates), *S. hominis* (1 isolate), *S. lugdunensis*/*haemolyticus*/*equorum* (1 isolate) and *S. saprophyticus* (1 isolate). Species from other genera were *T. solitarius* (2 isolates) and *T. koreensis* (1 isolate), *E. faecalis* (1 isolate) and *E. hirae* (1 isolate), and *C. divergens* (1

isolate). All staphylococcal species sequenced, with the only exception of *S. saprophyticus*, possessed a *Kpn*I site in the amplicon, whereas members of the other genera did not have this site. A first digestion with endonuclease *Kpn*I allowed us to classify isolates as belonging to the genus *Staphylococcus* or not. Out of the 227 isolates (Table 4), 218 isolates belonged to the genus *Staphylococcus* (96.0 %). Digestions with endonucleases *Mbo*I and *Ava*II resulted in three to four and two to three restriction fragments, respectively (Figure 1). The obtained patterns could be adscribed to the species *S. equorum*/*hominis*/*lugdunensis*, *S. epidermidis* or *S. pasteuri*/*warneri* (Figure 1). Amplicons digestible with AvaII were subjected to the action of endonuclease *Taq*I. Those with a *Taq*I site belonged to the species *S. hominis* (data not shown). *S. equorum*/*lugdunensis* was the most abundant species (144 isolates), followed by *S. epidermidis* (39 isolates) and *S. pasteuri*/*warneri* (25 isolates). The last two species were more frequently found in both untreated and HPP-treated Iberian ham samples at the beginning of the refrigeration period than in samples at the end of this period.

A few isolates, which were either digested with *Kpn*I but could not be digested with *Mbo*I or *Ava*II (six isolates) or could not be digested with *Kpn*I (three isolates), were sent to be sequenced. Those whith the *Kpn*I site were identified as *Kocuria palustris*, *Brachybacterium conglomeratum* and *Streptomyces flavofungini/lomondensis* (2 isolates each). Those without a *Kpn*I site turned out to be *Tetragenococcus halophilus*, *Enterococcus faecium* and *Kocuria rhizophila* (1 isolate each).

Eighteen of the 24 yeast isolates (Table 4) were identified as *Debaryomyces hansenii* (75.0 %). The remaining isolates were members of the genera *Moniliella* (2 isolates), *Rhodotorula* (2 isolates), *Cryptococcus* (1 isolate) and *Ustilago* (1 isolate).

4. Discussion

In the present study, microbial counts in individual samples of untreated Iberian ham ranged from 2.0 to 5.7 log cfu g^{-1} while average counts were close to 3 log cfu g^{-1} for all microbial groups. Those microbial levels were similar to counts reported for *Micrococcaceae* in Iberian ham (Rodríguez et al., 1994) and for aerobic mesophiles in Parma ham (Hinrichsen and Pedersen, 1995) and Serrano ham (de Alba et al., 2015; Martínez-Onandi et al., 2017a). They were lower than the microbial counts reported for entire Serrano ham, with counts of aerobic mesophiles ranging from 3 to 8 log cfu g^{-1} and of lactic acid bacteria from 2 to 6 log cfu g^{-1}, depending on sample location and time of post-salting (Blesa et al., 2008). Levels were also lower than the average counts (4.6 or 4.8 log cfu g^{-1}) found in vacuum-packaged Serrano ham slices (Clariana et al., 2011; Garriga et al., 2004), although in those works the differences could be partly explained by microbial contamination of ham during slicing and packaging.

Microorganisms, particularly staphylococci, are recognized to play an important role in the sensory characteristics of dry-cured ham through their enzymatic activities (Rodríguez et al., 1994; Vermassen et al., 2016). In fact, volatile compounds of microbial origin accounted for more than 6 % of the volatile fraction of untreated Iberian ham both at the beginning and the end of a prolonged refrigeration period (Martínez-Onandi et al., 2017b; 2017c). It has been reported that differences in ham chemical composition may affect microbial growth and metabolism (Martínez-Onandi et al., 2017a).

High a_w values are generally favourable for microbial growth and metabolism. However, the only microbial groups significantly affected in the present study were psychrotrophs and *Micrococcaceae*. Otherwise, low a_w values increase the resistance of microorganisms to HPP, as shown for *Staphylococcus aureus* and lactic acid bacteria after HPP at 600 MPa for 6 min in three meat products (marinated beef, cooked ham and dry-cured ham) of different a_w (Hugas et al., 2002). The lower a_w of Serrano ham seemed to

protect *L. monocytogenes* during HPP, resulting in higher survival than in Iberian ham immediately after treatment, but thereafter the lower a_w of Serrano ham appeared as less favourable for the recovery of injured cells and the viability of survivors (Morales et al., 2006). In the present study, counts of microbial groups in HPP-treated hams were not significantly influenced by a_w values. This result was in apparent contradiction with the fact that the volatile compounds derived from microbial metabolism seemed to be particularly affected in HPP-treated Iberian hams (Martínez-Onandi et al., 2017b; 2017c).

Salt is an essential element in the manufacturing process of dry-cured ham (Toldrá and Flores, 1998). It is the main source of *Micrococcaceae* in dry-cured ham and exerts a selective pressure on the types and levels of microorganisms (Cordero and Zumalacárregui, 2000). *Micrococcaceae* was the predominant microbial group in Parma ham (Hinrichsen and Pedersen, 1995) and Serrano ham (Carrascosa et al., 1988; Blesa et al., 2008) and, according to our results, *Micrococcaceae* was also the predominant microbial group in Iberian ham. Salt concentration did not influence any of the microbial groups in the present study, what might be explained by the narrow range of salt concentration in Iberian ham (3.02-5.62 % for the first group of hams and 3.51-5.35 % for the second group) in comparison with the wider range of salt concentration (2.87-7.91 %) found in Serrano ham (Martínez-Onandi et al., 2017a).

Intramuscular fat contributes to the flavour, odour and texture of dry-cured ham. It is especially important in the perception of juiciness in Iberian dry-cured ham since this product is strongly dehydrated (Lorido et al., 2015). Higher levels of aerobic mesophiles, *Micrococcaceae,* halophiles and yeasts in the fat than in the lean of Italian hams were found during the first 4 months at 8 °C, but those differences did not persist at the end of ripening (Giolitti et al., 1971). In the present study, higher levels of moulds and yeasts were recorded in HPP-treated hams of low and medium fat content at the end of the

refrigeration period. In Serrano ham, higher levels of aerobic mesophiles, psychrotrophs, and moulds and yeasts were recorded in untreated hams of low fat content than in those of medium or high fat content (Martínez-Onandi et al., 2017a).

Reductions in the counts of the different microbial groups shortly after the application of the HPP treatment ranged from 1.7 to 2.0 log cycles (Table 3), generally higher than the respective reductions reported for Serrano ham of similar microbial load, which ranged from 0.4 to 2.0 log cycles (Martínez-Onandi et al., 2017a). HPP treatment (600 MPa for 6 min) of sliced Serrano ham of higher microbial load resulted in a slightly higher reduction, of 2.6 log cfu g^{-1} (Clariana et al., 2011; Garriga et al., 2004). HPP causes microbial death due to a multiplicity of damage accumulated in different cell locations (Rendueles et al., 2011). Low a_w protects cells against pressure, but pressure-injured microorganisms are generally more sensitive to low a_w. In the present study, differences in microbial counts between untreated and HPP-treated Iberian ham samples diminished at the end of the 5-month refrigeration period (Table 3). Counts of aerobic mesophiles in sliced Serrano ham treated at 600 MPa were around 3 log cfu g^{-1} after storage at 4 °C for 4 months while the levels of lactic acid bacteria and yeasts remained under the detection limit (Garriga et al., 2004). The higher fat content and a_w of Iberian ham might be responsible for the differences between hams. In vacuum-packed "cecina", a dry-cured meat product, HPP treatment at 500 MPa for 5 min lowered counts of aerobic mesophiles, lactic acid bacteria, *Micrococcaceae*, moulds and yeasts by 2 log cfu g^{-1} and after 7 months at 6 °C the differences between untreated and HPP-treated samples ranged from 0.33 log cfu g^{-1} for lactic acid bacteria to 1.9 log cfu g^{-1} for aerobic mesophiles (Rubio et al., 2007).

Gram-positive catalase-positive cocci wereidentified as the predominant microorganisms along ripening of different types of dry-cured ham (Carrascosa et al.,

1988; Giolitti et al., 1971; Hinrichsen and Pedersen, 1995; Huerta et al., 1988). In the present study, 96.0 % of the identified isolates belong to the genus *Staphylococcus*, in agreement with previous reports for Iberian ham (Rodríguez et al., 1994) and "lacón", a dry-cured pork product (Lorenzo et al., 2012). In the cited works, about 70% of the isolates were *S. xylosus* and about 8% *S. equorum*, although the identification of isolates from these two species by biochemical methods is less reliable than by molecular methods (Blaiotta et al., 2014). When 56 staphylococcal isolates from Spanish dry-cured ham were identified by biochemical methods *S. xylosus* was the prevalent species (87.5 %) but sequencing of the *16S rDNA* resulted in the unambiguous identification of most strains (73.2 %) as belonging to *S. equorum* and 8.9 % of the strains to *S. vitulinus* (Landeta et al., 2011). In Serrano ham, DGGE permitted the identification of *S. equorum* and *S. succinus* (Martínez-Onandi et al., 2017a). In the present study, 63.4 % of all isolates were *S. equorum*, followed by *S. epidermidis* (17.2 %) and *S. pasteuri/warneri* (11.0 %). A few members of the family *Micrococcaceae* were found among the bacterial isolates (two *Kocuria palustris* and one *K. rhizophila*), in agreement with previous works on Iberian ham (Rodríguez et al., 1994), salt (Cordero & Zumalacárregui, 2000) and "lacón" (Lorenzo et al., 2012). *K. palustris* and *K. rhizophila* were first isolated from the rhizoplane of an aquatic plant (Kovács et al., 1999). *Brachybacterium conglomeratum* was previously known as *Micrococcus conglomeratus* (Takeuchi et al., 1995). Although this genus does not belong to the family *Micrococcaceae* but *Dermobacteraceae*, both families are members of the order *Micrococcales*. *Kocuria* isolates represented a 1.3% of all bacterial isolates and this percentage rised to 2.2 % when including the *Brachybacterium* isolates. Those percentages were similar to the ones previously found for *Micrococcaceae* in Iberian ham (1.4%; Rodríguez et al., 1996) or "lacón" (1.6-4%; Lorenzo et al., 2012).

This is the first time that members of the genera *Tetragenococcus* and *Carnobacterium* have been isolated from dry-cured ham. Although their contribution to dry-cured ham biochemical and sensory characteristics is not known, it is proven that they are able to adapt to the harsh conditions and survive throughout Iberian ham ripening.

The genus *Tetragenococcus* appeared in 1990 after reclassification of *Pediococcus halophilus* as *T. halophilus* (Collins et al., 1990). This is the only genus of lactic acid bacteria that can thrive at salt concentrations as high as 18 % NaCl and at high pH values (Röling and Verseveld, 1997). Three of the five species of this genus (*T. koreensis*, *T. halophilus* and *T. muriaticus*) are typically isolated from salt-rich foods and condiments such as kimchi, fish sauce, anchovy pickles and soy sauce. *T. koreensis* was first isolated from kimchi, a traditional vegetable-based fermented Korean food (Lee et al., 2005) and it has recently been isolated from a traditional Italian raw fermented sausage (Amadoro et al., 2015). *T. solitarius* was isolated from human ear secretions (Facklam & Collins, 1989). *T. koreensis* and *T. solitarius* are the only two species from this genus able to grow in MRS (Justé et al., 2012), as it was the case for our isolates. *T. halophilus* was isolated from MSA (containing 7.5% NaCl).

Carnobacterium species are able to grow in raw meat and meat products at temperatures close to 0 °C, and are among the dominant lactic acid bacteria in cooked meats (Samelis et al., 2000). However, this genus has not been associated with dry-cured ham and, as far as we know, its resistance to HPP treatment has not been reported. In the present study, the *C. divergens* isolate came from a HPP-treated Iberian ham sample and was able to grow on PCA at 8 °C.

Enterococci are present in many foods due to their ability to grow at low a_w, in the presence of 6.5 % NaCl, and at temperatures ranging from 10 to 45 °C. Although they can play a beneficial role in the ripening and aroma development of meat and dairy

products, enterococci have been associated with the spoilage of meat and meat products and the production of biogenic amines. Their presence in food is controversial since many strains can carry virulence and antibiotic resistance factors (Foulquié Moreno et al., 2006). They have been detected in several types of spoiled dry-cured ham (Marín et al., 1992). The resistance of a virulent *E. faecalis* strain in dry-cured ham to HPP was quite remarkable, with treatments of 750 MPa for at least 9.5 min being needed to achieve a 4 log-cycle reduction (Belletti et al., 2013). In the present study, the two *Enterococcus* isolates came from HPP-treated Iberian ham samples. Selected strains of the genus *Streptomyces* are used as starters in the manufacture of fermented sausages (Candogan et al., 2009).

D. hansenii can be found in many habitats with low aw, such as seawater, cheese, meat, wine, beer, fruit and soil as well as in high-sugar products. It is the most commonly isolated yeast in meat fermentations (Breuer & Harms, 2006). It has been isolated from surface samples of Iberian dry-cured ham together with *Candida zeylanoides* and other yeast species as *C. blankii*, *C. intermedia*, *Pichia carsonii* and *Rhodotorula rubra* (Núñez et al., 1996b). Its contribution to the generation of peptides and free amino acids from raw pork myofibrillar proteins (Martín et al., 2001; Rodríguez et al., 1998) and of volatile compounds as well as to the inhibition of lipid oxidation products during the ripening of dry-fermented sausages (Flores et al., 2004) has been studied. *D. hansenii* was also found to be part of Serrano ham yeast community (Martínez-Onandi et al., 2017a). Its isolation from both untreated and HPP-treated samples points to a certain degree of baroresistance.

5. Conclusions

Chemical composition had a moderate effect on Iberian ham microbiota, with a_w as the most influential parameter. High a_w hams had the highest counts of psychrotrophs and

Micrococcaceae. HPP treatment significantly reduced the levels of all the studied microbial groups and, although a recovery was observed during refrigerated storage, the levels in HPP-treated samples still remained 0.5 to 1.3 log cycles lower than in untreated samples after 5 months at 4 ºC. Concerning the biodiversity of Iberian ham microbiota, isolates belonging to the genus *Staphylococcus* represented 96.0 % of all isolates, with *S. equorum* as the most frequently isolated species. The presence of microorganisms not previously found in dry-cured ham, such as members of genera *Tetragenococcus* and *Carnobacterium*, here reported is noteworthy. *Debaryomyces hansenii*, representing 75.0 % of all yeast isolates, was the most frequently isolated yeast species. *Staphylococcus* and *Debaryomyces* isolates seemed to possess a certain degree of baroresistance, as shown by their presence in HPP-treated Iberian ham.

Acknowledgements

The authors wish to thank the Spanish Ministry of Science and Innovation for supporting the experimental work through project RTA2010-00029-C04-04, and INIA for the Ph. D. grant of N. Martínez-Onandi.

References

Andrés, A. I., Cava, R., Ventanas, J., Muriel, E., & Ruiz, J. (2004). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, 84, 375-381.

Amadoro, C., Rossi, F., Piccirilli, M., & Colavita, G. (2015). *Tetragenococcus koreensis* is part of the microbiota in a traditional Italian raw fermented sausage. *Food Microbiology*, 50, 78-82.

AOAC (2000). Official methods of analysis (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Armenteros, M., Aristoy, M. C., Barat, J. M., & Toldrá, F. (2009). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry*, 117, 627-633.

Belletti, N., Garriga, M., Aymerich, T., & Bover-Cid, S. (2013). High pressure inactivation of a virulent *Enterococcus faecalis* on dry-cured ham: Modeling the effect of processing parameters. *Innovative Food Science and Emerging Technologies*, 18, 43-47.

Blaiotta, G., Ercolini, D., Mauriello, G., Salzano, G., & Villani, F. (2004). Rapid and reliable identification of *Staphylococcus equorum* by a species-specific PCR assay targeting the *sodA* gene. *Systematic and Applied Microbiology*, 27, 696-702.

Blesa, E., Aliño, M., Barat, J.M., Grau, R., Toldrá, F., & Pagán, M.J. (2008). Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, 78, 135-142.

Breuer, U., & Harms, H. (2006). *Debaryomyces hansenii* - an extremophilic yeast with biotechnological potential. *Yeast*, 23, 415-437.

Campos G., Robles L., Alonso R., Nuñez M., Picon A. (2011). Microbial dynamics during the ripening of a mixed cow and goat milk cheese manufactured using frozen goat milk curds. *Journal of Dairy Science*, 94, 4766-4776.

Campus, M., Flores, M., Martinez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, 80, 1174-1181.

Candogan, K., Wardlaw, F.B., & Acton, J.C. (2009). Effect of starter culture on proteolytic changes during processing of fermented beef sausages. *Food Chemistry*, 116, 731-737.

Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, A., & García-Regueiro, J.A. (2011). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Science and Emerging Technologies*, 12, 456-465.

Collins, M.D., Williams, A.M., & Wallbanks, S. (1990). The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. *FEMS Microbiology Letters*, 70, 255-262.

Cordero, M.R., & Zumalacarregui, J.M. (2000). Characterization of *Micrococcaceae* isolated from salt used for Spanish dry-cured ham. *Letters in Applied Microbiology*, 31, 303-306.

Cornejo, I., Carrascosa, A.V., Marin, M.E., & Martin, P.J. (1992). Dry-cured ham-consideration about the origin of microorganisms that grow on the deep muscular tissues of dry-cured Spanish hams during processing. *Fleischwirtschaft*, 72, 1422-1425.

De Alba, M., Bravo, D., & Medina, M. (2015). Inactivation of *Listeria monocytogenes* and *Salmonella* Enteritidis in dry-cured ham by combined treatments of high pressure and the lactoperoxidase system or lactoferrin. *Innovative Food Science and Emerging Technologies*, 31, 54-59.

Facklam, R.R., & Collins, M.D. (1989). Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *Journal of Clinical Microbiology*, 27, 731-734.

Flores, M., Durá, M.A., Marco, A., & Toldra, F. (2004). Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science*, 68, 439-446.

Folch, J., Lees, M., & Sloane-Stanley, G.H.S. (1957). A simple method from the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.

Foulquié Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E., & De Vuyst, L. (2006). The role and application of enterococci in food and health. *International Journal of Food Microbiology*, 106, 1-24.

Garriga, M., Grèbol, N., Aymerich, M.T., Monfort, J.M., & Hugas, M. (2004). Microbial inactivation after high pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, 5, 451-457.

Giolitti, G., Cantoni, C., Bianchi, M., Renon, P., & Beretta, G. (1971). Microbiology and chemical changes in raw hams of Italian type. *Journal of Applied Microbiology*, 34, 51-61.

Guillamón, J.M., Sabaté, J., Barrio, E., Cano, J., & Querol, A. (1998). Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Archives of Microbiology*, 169, 387-392.

Hinrichsen, L.L., & Pedersen, S.B. (1995). Relationship among flavor, volatile compounds, chemical-changes, and microflora in Italian-type dry-cured ham during processing. *Journal of Agricultural and Food Chemistry*, 43, 2932-2940.

Huerta, T., Hernandez, J., Guamis, B., & Hernandez, E. (1988). Microbiological and physico-chemical aspects in dry-salted Spanish ham. *Zentralblatt für Mikrobiologie*, 143, 475-482.

Hugas, M., Garriga, M., & Monfort J.M. (2002). New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, 62, 359-71.

Justé, A., Van Trappen, S., Verreth, C., Cleenwerck, I., De Vos, P., Michiels, C.W., Lievens, B., & Willems, K.A. (2012). Characterization of *Tetragenococcus* strains from sugar thick juice reveals a new species, *Tetragenococcus osmophilus* sp. nov., and divides *Tetragenococcus halophilus* into two subspecies, *T. halophilus* subsp. *halophilus* subsp. nov. and *T. halophilus* subsp. *flandriensis* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 62, 129-137.

Kovács, G., Burghardt, J., Pradella, S., Schumann, P., Stackebrandt, E., & Màrialigeti, K. (1999). *Kocuria palustris* sp. nov, and *Kocuria rhizophila* sp. nov., isolated from the rhizoplane of the narrow-leaved cattail (*Typha angustifolia*). *International Journal of Systematic Bacteriology*, 49, 167-173.

Landeta, G., de las Rivas, B., Carrascosa, A.V., & Muñoz, R. (2007). Screening of biogenic amine production by coagulase-negative staphylococci isolated during industrial Spanish dry-cured ham processes. *Meat Science*, 77, 556-561.

Lee, M., Kim, M.K., Vancanneyt, M., Swings, J., Kim, S.H., Kang, M.S. & Lee, S.T. (2005). *Tetragenococcus koreensis* sp. nov., a novel rhamnolipid-producing bacterium. *International Journal of Systematic and Evolutionary Bacteriology*, 55, 1409-1413.

Lorenzo, J.M., Fontán, M.C.G., Gómez, M., Fonseca, S., Franco, I., & Carballo, J. (2012). Study of the *Micrococcaceae* and *Staphylococcaceae* throughout the manufacture of dry-cured Lacón (a Spanish traditional meat product) made without or with additives. *Journal of Food Research*, 1, 200-211.

Lorido, L., Estévez, M., Ventanas, J., & Ventanas, S. (2015). Salt and intramuscular fat modulate dynamic perception of flavour and texture in dry-cured hams. *Meat Science*, 107, 39-48.

Marín, M.E., de la Rosa, M.C., Carrascosa, A.V., Cornejo, I. (1992). Microbiological and physicochemical aspects of spoiled dry-cured Spanish hams. *Fleischwirtschaft*, 72, 1600-1605.

Martín, A., Córdoba, J.J., Rodríguez, M. M., Nuñez, F., & Asensio, M.A. (2001). Evaluation of microbial proteolysis in meat products by capillary electrophoresis. *Journal of Applied Microbiology*, 90, 163-171.

Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., & Picon, A. (2016). Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. *Meat Science*, 111, 130-138.

Martínez-Onandi, N., Castioni, A., San Martín, E., Rivas-Cañedo, A., Nuñez, M., Torriani, S., & Picon, A. (2017a). Microbiota of high-pressure-processed Serrano ham investigated by culture-dependent and culture-independent methods. *International Journal of Food Microbiology*, 241, 298-307.

Martinez-Onandi, N., Rivas-Cañedo, A., Ávila, M., Garde, S., Nuñez, M., & Picon, A. (2017b). Influence of physicochemical characteristics and high pressure processing on the volatile fraction of Iberian dry-cured ham. *Meat Science*, 131, 40-47.

Martínez-Onandi, N., Rivas-Cañedo, A., Picon, A., & Nuñez, M. (2017c). Influence of compositional characteristics, high pressure processing and prolonged refrigerated storage on the volatile compounds of Iberian dry-cured ham. *Meat Science*, (submitted).

Morales, P., Calzada, J., & Nuñez, M. (2006). Effect of high pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. *Journal of Food Prot*ection, 69, 2539-2543.

Núñez, F., Rodríguez, M.M., Bermúdez, E., Córdoba, J.J., & Asensio, M.A. (1996a). Composition and toxigenic potential of mould population on dry-cured Iberian ham. *International Journal of Food Microbiology*, 32, 531-533.

Núñez, F., Rodríguez, M.M., Córdoba, J.J., Bermúdez, M.E., & Asensio, M.A. (1996b). Yeast population during ripening of dry-cured Iberian ham. *International Journal of Food Microbiology*, 29, 271-280.

Rendueles, E., Omer, M.K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT-Food Science and Technology*, 44, 1251-1260.

Rodríguez, M., Núñez, F., Córdoba, J.J., Sanabria, C., Bermúdez, E., & Asensio, M.A. (1994). Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. *International Journal of Food Microbiology*, 85, 905-912.

Rodríguez, M., Núñez, F., Córdoba, J.J., Sanabria, C., Bermúdez, E., & Asensio, M.A. (1998). Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. *Journal of Applied Microbiology*, 85, 905-912.

Röling, W.F.M. & van Verseveld, H.W. (1997). Growth, maintenance and fermentation pattern of the salt-tolerant lactic acid bacterium *Tetragenococcus halophila* in anaerobic glucose limited retention cultures. *Antonie van Leeuwenhoek*, 72, 239-243.

Rubio, B., Martínez, B., García-Cachan, M.D., Rovira, J., & Jaime, I. (2007). Effect of high pressure preservation on the quality of dry cured beef "Cecina de León". *Innovative Food Science and Emerging Technologies*, 8, 102-110.

Ruiz, J., García, C., Muriel, E., Andrés, A.I., & Ventanas, J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science*, 61, 347-354.

Samelis, J., Kakouri, A., & Rementzis, J. (2000). Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4 °C. *Food Microbiology*, 17, 329-340.

Takeuchi, M., Fang, C.-X., & Yokota, A. (1995). Taxonomic Study of the Genus *Brachybacterium*: Proposal of *Brachybacterium conglomeratum* sp. nov., nom. rev., *Brachybacteriurn paraconglorneratum* sp. nov., and *Brachybacterium rhamnosum* sp. nov. *International Journal of Systematic Bacteriology* 45, 160-168.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, 38, 331-352.

Vermassen, A., Dordet-Frisoni, E., de La Foye, A., Micheau, P., Laroute, V., Leroy. S., & Talon, R. (2016). Adaptation of *Staphylococcus xylosus* to nutrients and osmotic stress in a salted meat model. *Frontiers in Microbiolology*, 7, 87.

Figure 1. Patterns obtained by partial amplified rDNA restriction analysis (ARDRA) with endonucleases *Kpn*I (1), *Mbo*I (2), *Ava*II (3). Undigested sample (4). *Staphylococcus equorum/hominis/lugdunensis* (a), *S. epidermidis* (b), and *S. pasteuri/warneri* (c)

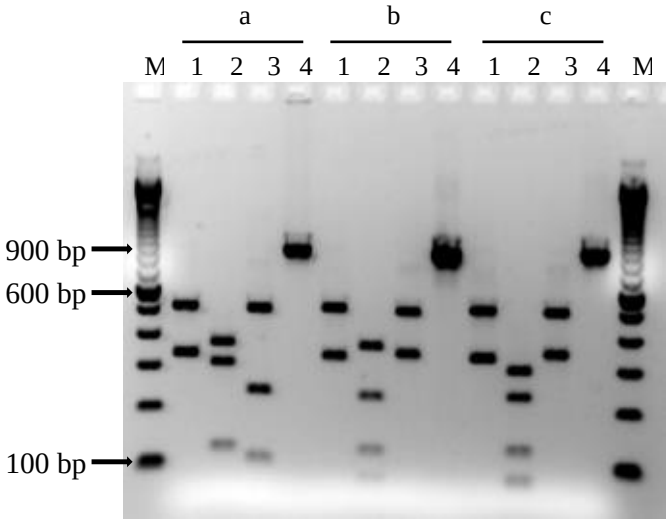


Table 1. Levels[1] of the main microbial groups in untreated and HPP-treated ripened Iberian hams as influenced by chemical composition, at the beginning of the 5-month refrigeration period.

Chemical parameter	Untreated ham				HPP-treated ham			
Microbial group	Low	Medium	High	P^2	Low	Medium	High	P^2
a_w[3]	(n = 6)	(n = 17)	(n = 7)		(n = 6)	(n = 17)	(n = 7)	
Aerobic mesophiles	2.82 ± 0.54	3.33 ± 0.92	3.46 ± 0.74	ns	1.55 ± 0.70	1.36 ± 0.59	1.73 ± 1.07	ns
Psychrotrophs	2.64 ± 0.74 [b]	3.19 ± 0.73 [ab]	3.39 ± 0.91 [a]	*	1.33 ± 0.49	1.09 ± 0.29	1.07 ± 0.27	ns
Lactic acid bacteria	2.59 ± 0.51	3.02 ± 0.78	2.84 ± 0.71	ns	0.76 ± 0.92	0.93 ± 0.78	0.73 ± 1.22	ns
Micrococcaceae	2.59 ± 0.75 [b]	3.21 ± 0.70 [a]	3.20 ± 0.57 [a]	*	1.08 ± 0.29	1.41 ± 0.54	1.43 ± 0.93	ns
Moulds and yeasts	2.92 ± 0.48	3.08 ± 0.45	3.14 ± 0.49	ns	1.33 ± 0.65	1.19 ± 0.50	1.07 ± 0.27	ns
Salt content[4]	(n = 11)	(n = 10)	(n = 9)		(n = 11)	(n = 10)	(n = 9)	
Aerobic mesophiles	3.53 ± 1.03	2.98 ± 0.83	3.23 ± 0.40	ns	1.52 ± 0.93	1.42 ± 0.61	1.51 ± 0.68	ns
Psychrotrophs	3.27 ± 1.04	2.94 ± 0.78	3.16 ± 0.43	ns	1.05 ± 0.21	1.10 ± 0.31	1.28 ± 0.46	ns
Lactic acid bacteria	3.03 ± 0.95	2.89 ± 0.65	2.73 ± 0.47	ns	1.15 ± 1.00	0.77 ± 0.69	0.57 ± 0.96	ns
Micrococcaceae	3.25 ± 0.60	2.90 ± 0.86	3.09 ± 0.67	ns	1.43 ± 0.82	1.40 ± 0.50	1.20 ± 0.46	ns
Moulds and yeasts	3.08 ± 0.66	2.97 ± 0.33	3.15 ± 0.28	ns	1.39 ± 0.70	1.10 ± 0.31	1.06 ± 0.24	ns
S/L ratio[5]	(n = 12)	(n = 9)	(n = 9)		(n = 12)	(n = 9)	(n = 9)	
Aerobic mesophiles	3.44 ± 1.03	3.04 ± 0.86	3.23 ± 0.40	ns	1.53 ± 0.91	1.40 ± 0.60	1.51 ± 0.68	ns
Psychrotrophs	3.17 ± 1.08	3.04 ± 0.69	3.16 ± 0.43	ns	1.04 ± 0.20	1.11 ± 0.32	1.28 ± 0.46	ns
Lactic acid bacteria	2.99 ± 0.93	2.93 ± 0.66	2.73 ± 0.47	ns	1.20 ± 0.93 [a]	0.66 ± 0.72 [b]	0.57 ± 0.96 [b]	*
Micrococcaceae	3.18 ± 0.65	2.96 ± 0.86	3.09 ± 0.67	ns	1.44 ± 0.79	1.39 ± 0.50	1.20 ± 0.46	ns
Moulds and yeasts	3.12 ± 0.56	2.90 ± 0.46	3.15 ± 0.28	ns	1.23 ± 0.56	1.28 ± 0.57	1.06 ± 0.24	ns
IMF content[6]	(n = 9)	(n = 10)	(n = 11)		(n = 9)	(n = 10)	(n = 11)	
Aerobic mesophiles	3.18 ± 0.69	3.18 ± 0.92	3.40 ± 0.88	ns	1.42 ± 0.63	1.40 ± 0.94	1.62 ± 0.66	ns
Psychrotrophs	2.93 ± 0.98	3.29 ± 0.81	3.14 ± 0.64	ns	1.06 ± 0.24	1.15 ± 0.37	1.18 ± 0.39	ns
Lactic acid bacteria	2.81 ± 0.67	2.96 ± 0.66	2.90 ± 0.86	ns	0.77 ± 0.76	0.78 ± 1.08	0.97 ± 0.90	ns
Micrococcaceae	3.17 ± 0.75	3.05 ± 0.72	3.04 ± 0.72	ns	1.28 ± 0.46	1.40 ± 0.82	1.37 ± 0.56	ns
Moulds and yeasts	2.99 ± 0.30	3.14 ± 0.47	3.05 ± 0.57	ns	1.06 ± 0.24	1.10 ± 0.31	1.39 ± 0.70	ns

[1]Levels are expressed in log cfu g^{-1}, mean ± SD of duplicate determinations.

[2]Significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[3]Low a_w was < 0.868, medium a_w was within the range 0.868-0.883, and high a_w was > 0.883.

[4]Low salt content was < 3.93 %, medium salt content was within the range 3.93-4.60 %, and high salt content was

> 4.60 %.

[5]Low salt-in-lean (S/L) ratio was < 0.045, medium S/L ratio was within the range 0.045-0.052, and high S/L ratio was > 0.052.

[6]Low intramuscular fat (IMF) content was < 10.18 %, medium IMF content was within the range 10.18-13.75 %, and high IMF content was > 13.75 %.

Table 2. Levels[1] of the main microbial groups in untreated and HPP-treated ripened Iberian hams as influenced by chemical composition, at the end of the 5-month refrigeration period

Chemical parameter	Untreated ham				HPP-treated ham			
Microbial group	Low	Medium	High	P^2	Low	Medium	High	P^2
a_w[3]	(n = 11)	(n = 9)	(n = 10)		(n = 11)	(n = 9)	(n = 10)	
Aerobic mesophiles	2.99 ± 1.25	2.94 ± 0.68	2.75 ± 0.51	ns	2.41 ± 1.16	2.13 ± 0.92	2.53 ± 1.01	ns
Psychrotrophs	2.97 ± 1.08	2.97 ± 0.84	3.32 ± 0.74	ns	2.39 ± 1.26	1.89 ± 0.84	1.89 ± 1.03	ns
Lactic acid bacteria	3.32 ± 1.14	3.29 ± 0.63	3.23 ± 0.89	ns	1.98 ± 1.44	1.50 ± 1.20	2.28 ± 1.00	ns
Micrococcaceae	2.82 ± 1.30	2.88 ± 0.54	2.73 ± 1.25	ns	1.92 ± 1.19	1.62 ± 0.93	2.07 ± 1.14	ns
Moulds and yeasts	2.64 ± 0.89	2.69 ± 0.58	2.94 ± 0.73	ns	2.22 ± 1.22	1.66 ± 0.90	1.87 ± 0.70	ns
Salt content[4]	(n = 12)	(n = 7)	(n = 11)		(n = 12)	(n = 7)	(n = 11)	
Aerobic mesophiles	2.64 ± 0.60	3.19 ± 0.40	2.99 ± 1.25	ns	2.35 ± 1.05	2.32 ± 0.86	2.41 ± 1.16	ns
Psychrotrophs	3.17 ± 0.86	3.14 ± 0.71	2.97 ± 1.08	ns	1.83 ± 0.97	2.00 ± 0.89	2.39 ± 1.26	ns
Lactic acid bacteria	3.18 ± 0.83	3.38 ± 0.65	3.32 ± 1.14	ns	2.00 ± 1.14	1.76 ± 1.20	1.98 ± 1.44	ns
Micrococcaceae	2.69 ± 1.15	3.00 ± 0.53	2.82 ± 1.30	ns	1.89 ± 1.11	1.80 ± 0.99	1.92 ± 1.19	ns
Moulds and yeasts	2.81 ± 0.73	2.84 ± 0.56	2.64 ± 0.89	ns	1.72 ± 0.72	1.85 ± 0.94	2.22 ± 1.22	ns
S/L ratio[5]	(n = 10)	(n = 9)	(n = 11)		(n = 10)	(n = 9)	(n = 11)	
Aerobic mesophiles	2.75 ± 0.51	2.94 ± 0.68	2.99 ± 1.25	ns	2.53 ± 1.01	2.13 ± 0.92	2.41 ± 1.16	ns
Psychrotrophs	3.32 ± 0.74	2.97 ± 0.84	2.97 ± 1.08	ns	1.89 ± 1.03	1.89 ± 0.84	2.39 ± 1.26	ns
Lactic acid bacteria	3.23 ± 0.89	3.29 ± 0.63	3.32 ± 1.14	ns	2.28 ± 1.00	1.50 ± 1.20	1.98 ± 1.44	ns
Micrococcaceae	2.73 ± 1.25	2.88 ± 0.54	2.82 ± 1.30	ns	2.07 ± 1.14	1.62 ± 0.93	1.92 ± 1.19	ns
Moulds and yeasts	2.94 ± 0.73	2.69 ± 0.58	2.64 ± 0.89	ns	1.87 ± 0.70	1.66 ± 0.90	2.22 ± 1.22	ns
IMF content[6]	(n = 10)	(n = 11)	(n = 9)		(n = 10)	(n = 11)	(n = 9)	
Aerobic mesophiles	2.58 ± 0.94	3.19 ± 0.91	2.89 ± 0.68	ns	2.27 ± 1.03	2.52 ± 1.04	2.28 ± 1.08	ns
Psychrotrophs	3.13 ± 0.70	2.99 ± 1.08	3.16 ± 0.91	ns	1.88 ± 1.09	2.35 ± 1.11	1.95 ± 1.03	ns
Lactic acid bacteria	3.36 ± 1.08	3.40 ± 1.05	3.05 ± 0.37	ns	1.90 ± 1.32	2.11 ± 1.31	1.76 ± 1.14	ns
Micrococcaceae	2.80 ± 1.39	2.86 ± 1.11	2.75 ± 0.68	ns	1.80 ± 1.03	2.03 ± 1.19	1.80 ± 1.10	ns
Moulds and yeasts	2.59 ± 0.70	2.82 ± 0.76	2.85 ± 0.80	ns	2.13 ± 1.08 [a]	2.15 ± 1.02 [a]	1.46 ± 0.70 [b]	*

[1]Levels are expressed in log cfu g^{-1}, mean ± SD of duplicate determinations.

[2]Significance in the analysis of variance: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[3]Low a_w was < 0.871, medium a_w was within the range 0.871-0.879, and high a_w was > 0.879.

[4]Low salt content was < 4.23 %, medium salt content was within the range 4.23-4.72 %, and high salt content was > 4.72 %.

[5]Low salt-in-lean (S/L) ratio was < 0.048, medium S/L ratio was within the range 0.048-0.054, and high S/L ratio was > 0.054.

[6]Low intramuscular fat (IMF) content was < 11.32 %, medium IMF content was within the range 11.32-13.44 %, and high IMF content was > 13.44 %.

Table 3. Levels[1] of the main microbial groups in untreated and HPP-treated ripened Iberian hams as influenced by HPP treatment and refrigerated storage at 4 ºC for 5 months.

Microbial group[2]	Refrigerated storage	Untreated hams (n = 30)	HPP-treated hams (n = 30)
Aerobic mesophiles	0 months	3.26 ± 0.84 ^{aA}	1.48 ± 0.75 ^{bB}
	5 months	2.90 ± 0.88 ^{aB}	2.37 ± 1.04 ^{bA}
Psychrotrophs	0 months	3.13 ± 0.81 ^{aA}	1.13 ± 0.34 ^{bB}
	5 months	3.09 ± 0.90 ^{aA}	2.07 ± 1.08 ^{bA}
Lactic acid bacteria	0 months	2.89 ± 0.73 ^{aB}	0.85 ± 0.92 ^{bB}
	5 months	3.28 ± 0.91 ^{aA}	1.94 ± 1.25 ^{bA}
Micrococcaceae	0 months	3.09 ± 0.72 ^{aA}	1.35 ± 0.62 ^{bB}
	5 months	2.81 ± 1.09 ^{aA}	1.88 ± 1.10 ^{bA}
Moulds and yeasts	0 months	3.06 ± 0.47 ^{aA}	1.19 ± 0.49 ^{bB}
	5 months	2.75 ± 0.75 ^{aB}	1.94 ± 0.99 ^{bA}

[1]Levels are expressed in log cfu g^{-1}, mean ± SD of duplicate determinations.

[2]Means within the same row with different lower-case superscripts differ significantly at $P < 0.01$. Means for the same microbial group within the same column with different upper-case superscripts differ significantly at $P < 0.01$.

Table 4. Identification at the genus and species level of 227 bacterial isolates from untreated and HPP-treated Iberian hams at the beginning and the end of the 5-month refrigeration period.

Time	Ham	Genus	Species	No.
0 months	Untreated	*Staphylococcus*	*equorum/lugdunensis*	38
			epidermidis	13
			pasteuri/warneri	8
			saprophyticus	1
		Debaryomyces	*hansenii*	2
		Moniliella	*nigrescens*	2
	HPP-treated	*Staphylococcus*	*equorum/lugdunensis*	12
			epidermidis	23
			hominis	1
			pasteuri/warneri	13
		Enterococcus	*faecalis*	1
			faecium/hirae	2
		Carnobacterium	*divergens*	1
		Debaryomyces	*hansenii*	3
		Cryptococcus	*magnus*	1
5 months	Untreated	*Staphylococcus*	*equorum/lugdunensis*	46
			epidermidis	3
			hominis	2
			pasteuri/warneri	4
		Kocuria	*palustris*	2
		Tetragenococcus	*halophilus*	1
			koreensis	1
			solitarius	2
		Debaryomyces	*hansenii*	5
	HPP-treated	*Staphylococcus*	*equorum/lugdunensis*	48
		Kocuria	*rhizophila*	1
		Brachybacterium	*conglomeratum*	2
		Streptomyces	*flavofungini/lomodensis*	2
		Debaryomyces	*hansenii*	8
		Rhodotorula	*mucilaginosa*	2
		Ustilago	*cynodontis/sparsa*	1

8. Discusión integradora

8.1. Compuestos volátiles identificados en jamón Serrano y jamón Ibérico

En la fracción volátil del **jamón Serrano** se identificaron 100 compuestos al principio del periodo de refrigeración, tanto en las muestras control como en las muestras tratadas por APH. La fracción volátil incluía 7 ácidos, 21 alcoholes, 7 aldehídos, 6 alcanos, 5 ésteres, 10 cetonas, 20 compuestos bencénicos, 7 compuestos azufrados, 6 furanos, 2 furanonas, 3 pirazinas, 2 terpenos y 4 compuestos misceláneos. Al final del periodo de 5 meses en refrigeración se identificaron 103 compuestos volátiles en el jamón Serrano. Su fracción volátil incluía 8 ácidos, 21 alcoholes, 7 aldehídos, 8 alcanos, 6 ésteres, 9 cetonas, 18 compuestos bencénicos, 7 compuestos azufrados, 7 furanos, 2 furanonas, 4 pirazinas, 2 terpenos y 4 compuestos misceláneos.

El número de compuestos identificados en jamón Serrano en la presente Tesis, después de una extracción de las muestras por SPME, es algo más elevado que los 84 a 93 compuestos identificados en jamón Serrano en trabajos anteriores, después de una extracción de las muestras mediante espacio de cabeza dinámico de purga y trampa (Flores et al., 1997; Rivas-Cañedo et al., 2009a; Sabio et al., 1998).

Por otra parte, en la fracción volátil del **jamón Ibérico** se identificaron 122 compuestos al principio del periodo de refrigeración, tanto en las muestras control como en las muestras tratadas por APH. La fracción volátil incluía 10 ácidos, 18 alcoholes, 11 aldehídos, 18 cetonas, 10 ésteres, 13 alcanos, 23 compuestos bencénicos, 5 compuestos azufrados, 4 furanos, 5 furanonas, 3 pirazinas y 2 compuestos misceláneos. Al final del periodo de 5 meses en refrigeración se identificaron 116 compuestos volátiles en jamón Ibérico. Su fracción volátil incluía 11 ácidos, 19 alcoholes, 13 aldehídos, 16 cetonas, 7 ésteres, 9 alcanos, 21 compuestos bencénicos, 5 compuestos azufrados, 4 furanos, 5 furanonas, 3 pirazinas y 3 compuestos misceláneos.

El número de compuestos identificados en jamón Ibérico en la presente Tesis, después de una extracción de las muestras por SPME, es superior a los 55 a 109 compuestos identificados en jamón Ibérico en trabajos anteriores considerados individualmente, después de una extracción de las muestras

mediante espacio de cabeza dinámico de purga y trampa o mediante SPME (García et al., 1991; López et al., 1992; Narváez-Rivas et al., 2010; Ramírez & Cava, 2007; Ruiz et al., 1998; Ruiz et al., 1999; Sánchez-Peña et al., 2005; Timón et al., 2001). Sin embargo, el número total de compuestos volátiles identificados en jamón Ibérico en el conjunto de los trabajos anteriores asciende a 411 (Narváez-Rivas et al., 2012).

El mayor contenido en grasa intramuscular del jamón Ibérico puede ser, al menos en parte, responsable de su mayor número de compuestos volátiles en comparación con el jamón Serrano al final de la maduración, es decir, al principio del periodo de refrigeración. Se ha comprobado que el contenido en grasa intramuscular influye sobre la oxidación de los lípidos y las características sensoriales del jamón Ibérico (Fuentes et al., 2014a; Fuentes et al., 2014b). Con independencia de su composición, otros factores tales como la temperatura de maduración afectan a la formación y estabilidad de los compuestos volátiles del jamón curado (Andres et al., 2005).

8.2. Efecto de la composición del jamón curado sobre los compuestos volátiles

Las principales características de composición de los jamones Serrano e Ibérico estudiados en la presente Tesis se recogen en la Tabla 8.1.

Tabla 8.1. Grasa intramuscular, sal, relación sal en magro y actividad de agua de los jamones Serrano e Ibérico estudiados en la presente Tesis

Variable	Tiempo	Jamón Serrano			Jamón Ibérico		
		Intervalo	Media ± DS	*P*	Intervalo	Media ± DS	P
Grasa intramuscular, %	0 meses	1,83-14,03	5,28 ± 3,45		4,63-18, 59	11,96 ± 3,56	
	5 meses	2,28-12,47	7,01 ± 2,40	*	8,87-16, 62	12,38 ± 2,12	NS
Sal, %	0 meses	2,87-7,91	5,49 ± 1,30		3,02-5,62	4,27 ± 0,67	
	5 meses	3,21-6,77	4,41 ± 0,83	***	3,51-5,35	4,48 ± 0,50	NS
Sal en magro	0 meses	0,033-0,081	0,058 ± 0,013		0,036-0,063	0,049 ± 0,008	
	5 meses	0,035-0,069	0,047 ± 0,009	***	0,042-0,061	0,051 ± 0,005	NS
Actividad de agua	0 meses	0,833-0,883	0,859 ± 0,013		0,845-0,912	0,876 ± 0,015	
	5 meses	0,860-0,899	0,880 ± 0,010	***	0,859-0,890	0,875 ± 0,008	NS

P entre tiempos de refrigeración: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significativo.

8.2.1. Efecto del contenido en grasa intramuscular del jamón curado sobre los compuestos volátiles

La contribución de la grasa y de la lipolisis al sabor y el aroma del jamón curado es fundamental. Los ácidos grasos libres generados por la acción de las lipasas y las fosfolipasas se oxidan formando aldehídos, alcanos, alcoholes, cetonas, ésteres y otros compuestos volátiles mediante reacciones químicas de auto-oxidación o reacciones enzimáticas de β-oxidación (Toldrá & Flores, 1998). Además, el jamón curado sufre una fuerte deshidratación durante el proceso de elaboración, por lo que la grasa intramuscular juega un papel importante en la jugosidad del producto (Ruíz et al., 1998).

El **contenido en grasa intramuscular del jamón Serrano**, influyó sobre un número elevado de compuestos volátiles, 28 al principio de la refrigeración y 46 al final de la refrigeración. Al principio del periodo de refrigeración del jamón Serrano, el contenido de grasa intramuscular afectó en los jamones control a los niveles de algunos ácidos, alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanos y pirazinas mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos, alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, ésteres, furanos, furanonas y pirazinas. Los jamones con contenidos altos de grasa intramuscular mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica (ácido propanoico, ácido hexanoico, ácido octanoico, fenol, 3-etilfenol y 3-fenoxi-1-propanol), de reacciones de Maillard (2-metilfurano, 2,5-dimetilfurano y 2,6-dimetilpirazina), de la reacción de Strecker (2-metilbutanal) y del catabolismo de aminoácidos azufrados (trisulfuro de dimetilo) mientras que los jamones con contenidos bajos de grasa intramuscular mostraron niveles elevados de compuestos procedentes del metabolismo microbiano (2-propanol, 2-butanol, 2-pentanol, 2-metil-2-buten-1-ol, 2-butoxietanol, 2-(2-etoxietoxi)-etanol y 1-metoxi-2-propanol). Por lo que respecta a las cetonas (2,3-pentanodiona y 4-metil-2-pentanona) no se observó una tendencia definida, lo que podría

ser debido a que las cetonas tienen distintas rutas de formación, bien sea por oxidación lipídica o por β-oxidación de ácidos grasos llevada a cabo por mohos en la superficie del jamón.

Al final del periodo de refrigeración del jamón Serrano, el contenido de grasa intramuscular afectó a los niveles de algunos ácidos, alcoholes, aldehídos, alcanos, cetonas, compuestos bencénicos, compuestos azufrados, ésteres, furanos y pirazinas mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos, alcoholes, aldehídos, alcanos, cetonas, compuestos bencénicos, compuestos azufrados, ésteres, furanos y furanonas. En líneas generales, los resultados observados fueron similares a los obtenidos al inicio del periodo de refrigeración. Los jamones con contenidos altos de grasa intramuscular mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica (ácido hexanoico, ácido heptanoico, hexanal, fenol, 3-etilfenol y 3-fenoxi-1-propanol), de reacciones de Maillard (2-metilfurano, 2-etilfurano, 2,5-dimetilfurano y 2,6-dimetilpirazina) y de la reacción de esterificación enzimática (butanoato de etilo, hexanoato de etilo y decanoato de etilo) mientras que los jamones con contenidos bajos de grasa intramuscular tenían niveles elevados de compuestos procedentes del metabolismo microbiano (2-propanol, 2-butoxietanol y 1-metoxi-2-propanol). Sin embargo, los compuestos azufrados (disulfuro de dimetilo y trisulfuro de dimetilo) no mostraron una tendencia definida. Los jamones con contenidos bajos de grasa intramuscular mostraron niveles elevados de sulfuro de dimetilo mientras que los jamones con contenidos medios de grasa intramuscular tenían niveles elevados de trisulfuro de dimetilo.

El **contenido en grasa intramuscular del jamón Ibérico**, tuvo una influencia mucho menor sobre los compuestos volátiles a pesar de su mayor contenido en grasa, afectando a 20 compuestos al principio del periodo de refrigeración y a únicamente 5 compuestos al final de dicho periodo. Al principio de la refrigeración, en los jamones control el contenido de grasa intramuscular afectó a los niveles de algunos alcoholes, cetonas, compuestos bencénicos y furanonas mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos, aldehídos, cetonas, compuestos bencénicos y compuestos azufrados. Los jamones Ibéricos con contenidos medios y altos de grasa intramuscular

mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica (1-(2-furanil)-etanona, etilfenol y *m*-etiltolueno) y de compuestos procedentes de la dieta de los animales (*o*-xileno, *m*-xileno y *p*-xileno).

Al final del periodo de refrigeración del jamón Ibérico, en los jamones control el contenido de grasa intramuscular afectó a los niveles de algunos alcoholes, aldehídos y compuestos bencénicos mientras que en los jamones tratados por APH solo afectó a los niveles de algunos alcanos.

8.2.2. Efecto de la concentración de sal y la actividad de agua del jamón curado sobre los compuestos volátiles

La sal es un ingrediente fundamental en la elaboración del jamón curado. Con el salado se pretende que el pernil adquiera un contenido en sal suficiente para que, una vez distribuido por toda la pieza y en combinación con la paulatina deshidratación, inhiba el desarrollo de microorganismos alterantes y potencialmente patógenos para el consumidor y permita la obtención de un producto con un ligero sabor salado. La sal puede regular la actividad enzimática endógena en el jamón curado y las reacciones químicas durante la maduración (Motilva & Toldrá, 1993). Sin embargo, en un estudio sobre la fracción volátil de jamones Ibéricos de diferente contenido en sal (Ventanas et al., 2008) se concluyó que el único compuesto afectado por la concentración de sal era el 2-pentilfurano, que mostraba niveles más bajos en los jamones con elevada concentración de sal.

La **concentración de sal del jamón Serrano** influyó sobre un número considerable de compuestos volátiles en el presente estudio, afectando a 23 compuestos al principio de la refrigeración y a 7 compuestos al final de la refrigeración. Al principio del periodo de refrigeración, la concentración de sal de los jamones control afectó a algunos alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanos y pirazinas mientras que en los jamones tratados por APH afectó a algunos alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanos y pirazinas. No se registró una influencia de la concentración de sal sobre los aldehídos lineales,

originados mediante reacciones de oxidación lipídica. Sin embargo, jamones con baja concentración de sal mostraron niveles elevados de 2-metilbutanal cuya formación se vio probablemente favorecida por el incremento de la concentración de aminoácidos libres como consecuencia de una mayor proteolisis. Los jamones con alta concentración de sal mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica o de la β-oxidación de ácidos grasos libres por mohos (1-butanol, 1-pentanol, 1-octanol, 1-penten-3-ol, 3-hidroxi-2-butanona y 4-metil-2-pentanona) y de compuestos procedentes del metabolismo microbiano (2-butanol, 2-pentanol, 2-metil-2-buten-1-ol y 2-butoxietanol) mientras que los jamones con baja concentración de sal mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (2-metilfurano, metilpirazina y 2,6-dimetilpirazina).

Al final del periodo de refrigeración del jamón Serrano, la concentración de sal afectó en los jamones control a los niveles de algunos alcoholes, cetonas y pirazinas mientras que en los jamones tratados por APH afectó a los niveles de algunos compuestos azufrados y pirazinas. Los jamones con baja concentración de sal mostraron niveles elevados de metilpirazina.

La **concentración de sal del jamón Ibérico** influyó sobre un menor número de compuestos volátiles, afectando a 2 compuestos al principio de la refrigeración y a 5 compuestos al final de la refrigeración. Al principio del periodo de refrigeración, la concentración de sal de los jamones control afectó a los niveles de algunos aldehídos y cetonas mientras que en los jamones tratados por APH ningún compuesto resultó afectado. Los jamones con baja concentración de sal mostraron niveles elevados de compuestos procedentes de la oxidación lipídica (etanal y 2,3-pentanodiona).

Al final del periodo de refrigeración del jamón Ibérico, la concentración de sal en los jamones control afectó a los niveles de algunos ácidos y compuestos bencénicos mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos y alcoholes. Los jamones con valores medios de concentración de sal mostraron niveles elevados de compuestos presumiblemente procedentes del metabolismo microbiano (ácido acético y ácido propanoico) y de reacciones de oxidación lipídica

(ácido nonanoico y feniletanal) mientras que los jamones con baja concentración de sal mostraron niveles elevados de 3-metil-1-butanol, compuesto de origen microbiano. Diferentes estudios (Andrés et al., 2007; Armenteros et al., 2012; Wang et al., 2012) han mostrado que tanto las variaciones en la concentración de sal como la sustitución de la sal común por otras sales influían en la formación de algunos compuestos volátiles durante la maduración del jamón, probablemente debido a la influencia de la sal en las reacciones de oxidación lipídica y proteolisis, las cuales parecen depender del músculo dentro de un mismo jamón (Andrés et al., 2007).

La **relación sal en magro del jamón Serrano** influyó sobre un número considerable de compuestos volátiles, afectando a afectando a 18 compuestos al principio de la refrigeración y a 7 compuestos al final de la refrigeración. Al principio del periodo de refrigeración, la relación sal en magro de los jamones control afectó a los niveles de algunos alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados y pirazinas mientras que en los jamones tratados por APH afectó a los niveles de algunos alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados y pirazinas. En jamón Serrano, los resultados obtenidos para la relación sal en magro son muy similares a los obtenidos para la concentración de sal. Jamones con valores altos de la relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica o de β-oxidación de ácidos grasos libres por mohos (1-butanol, 1-pentanol, 1-octanol, 1-penten-3-ol, 3-hidroxi-2-butanona y 4-metil-2-pentanona) y de compuestos procedentes del metabolismo microbiano (2-metil-2-buten-1-ol y 2-butoxietanol) mientras que jamones con valores bajos de la relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (metilpirazina y 2,6-dimetilpirazina) y de la degradación de Strecker (2-metilbutanal).

Al final del periodo de refrigeración del jamón Serrano, la relación sal en magro en los jamones control afectó a los niveles de algunos alcoholes y pirazinas mientras que en los jamones tratados por APH afectó a los niveles de algunos compuestos azufrados y pirazinas. Los jamones con valores bajos

de la relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (metilpirazina y 2,6-dimetilpirazina).

La **relación sal en magro del jamón Ibérico** tuvo una influencia mucho menor sobre los compuestos volátiles, no afectando a ningún compuesto al principio de la refrigeración y a únicamente 4 compuestos al final de la refrigeración.

Al final del periodo de refrigeración del jamón Ibérico, la relación sal en magro de los jamones control afectó a los niveles de algunos ácidos y aldehídos mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos, alcoholes y alcanos. Los jamones con valores medios y bajos de la relación sal en magro mostraron niveles elevados de compuestos presumiblemente procedentes del metabolismo microbiano (ácido acético y 3-metil-1-butanol) mientras que únicamente los jamones con valores bajos de la relación sal en magro mostraron niveles elevados de compuestos procedentes de la oxidación lipídica (etanal).

La actividad de agua del jamón curado puede influir en la formación de compuestos volátiles durante su procesado, maduración y almacenamiento a través de su efecto sobre la actividad de microorganismos y enzimas. El efecto de la a_w sobre la actividad de los microorganismos en el jamón curado es difícil de evaluar (Hinrichsen & Pedersen, 1995), aunque por lo general valores elevados de a_w favorecen tanto el crecimiento como el metabolismo microbiano. El efecto de variaciones en los niveles de la a_w sobre la actividad enzimática en la carne de cerdo no es uniforme (Motilva et al., 1992; Toldrá et al., 1993; Toldrá, 2006). La disminución de los valores de la a_w provoca una disminución de la actividad de las enzimas lipasas neutras y básicas, un aumento de la actividad de la lipasa ácida, una ligera inactivación de la esterasa neutra y una ligera activación de la esterasa ácida.

La **actividad de agua del jamón Serrano** influyó sobre los niveles de un número considerable de compuestos volátiles, 13 compuestos al principio de la refrigeración y 12 al final de la refrigeración. Al principio del periodo de refrigeración, la a_w influyó sobre los niveles de algunos ácidos, alcoholes, cetonas, compuestos bencénicos, compuestos azufrados, ésteres, furanos y furanonas tanto en los

jamones control como en los tratados por APH. Los jamones con valores altos de a_w mostraron niveles elevados de compuestos volátiles procedentes del metabolismo microbiano (etanol, 2-propanol, 1-metoxi-2-propanol, acetato de etilo y sulfuro de dimetilo) mientras que los jamones con valores bajos de a_w tenían niveles elevados de compuestos volátiles procedentes de la actividad enzimática.

Al final del periodo de refrigeración del jamón Serrano, la actividad de agua afectó a los niveles de algunos alcoholes y alcanos en los jamones control mientras que en los jamones tratados por APH afectó a los niveles de algunos alcoholes, aldehídos, cetonas, compuestos bencénicos y compuestos azufrados. Los jamones con valores medios de a_w mostraron niveles elevados de alguno de los compuestos procedentes del metabolismo microbiano (2-pentanol).

La **actividad de agua del jamón Ibérico** influyó en un menor número de compuestos volátiles, únicamente 5 compuestos al principio de la refrigeración y 4 compuestos al final de la refrigeración. Al principio del periodo de refrigeración, la a_w afectó a los niveles de algunos alcoholes y aldehídos en los jamones control mientras que en los jamones tratados por APH afectó a los niveles de algunos alcoholes y compuestos azufrados. Jamones con valores altos de a_w mostraron niveles elevados de compuestos procedentes del metabolismo microbiano (etanol).

Al final del periodo de refrigeración del jamón Ibérico, la a_w afectó a los niveles de algunos ácidos y aldehídos en los jamones control mientras que en los jamones tratados por APH afectó a los niveles de algunos ácidos, alcoholes y alcanos. Los jamones con valores medios de a_w tenían niveles elevados de compuestos procedentes del metabolismo microbiano (ácido acético).

La variable de composición que más influyó sobre la fracción volátil tanto del jamón Serrano como del jamón Ibérico fue el contenido de grasa intramuscular. El contenido en grasa intramuscular influyó principalmente sobre los compuestos volátiles que se generan a partir de reacciones de oxidación lipídica como son los alcoholes, cuyo aroma es muy agradable y proporciona notas de olores frescos y tostados (Toldrá & Flores, 1998), los aldehídos lineales, cuyo aroma proporciona notas cárnicas (Shahidi et al., 1986) y los compuestos bencénicos y, en menor medida, sobre los compuestos

que provienen de reacciones de Maillard como son las pirazinas, que proporcionan olores a nuez, asado o tostado (Barbieri et al., 1992), los compuestos procedentes de la degradación de Strecker como son los aldehídos ramificados, que proporcionan los aromas característicos a curado (Dumont & Adda, 1972), los compuestos procedentes del metabolismo microbiano como son los 2-alcoholes y los compuestos procedentes de la esterificación enzimática como son los ésteres, que imparten notas frutales (Stahnke, 1995).

La concentración de sal y la relación sal en magro influyeron principalmente sobre los compuestos volátiles que se generan a partir de reacciones de oxidación lipídica, de β-oxidación de ácidos grasos libres por mohos, del metabolismo microbiano y, en menor medida, de reacciones de Maillard.

Los valores de a_w influyeron sobre los compuestos derivados del metabolismo microbiano y, en menor medida, sobre los compuestos procedentes de reacciones de Maillard y de la degradación de Strecker.

8.3. Efecto del tratamiento por altas presiones del jamón curado sobre los compuestos volátiles

En **jamón Serrano** al principio del periodo de refrigeración, únicamente 8 de los 100 compuestos volátiles identificados resultaron afectados significativamente por el tratamiento de APH. Dos compuestos azufrados (metanotiol y dióxido de sulfuro) alcanzaron mayores niveles en los jamones Serranos tratados por APH mientras que 4 ésteres (acetato de etilo, butanoato de etilo, 2-metilbutanoato de etilo y 3-metilbutanoato de etilo) y 2 compuestos azufrados (disulfuro de dimetilo y trisulfuro de dimetilo) alcanzaron menores niveles en los jamones Serrano tratados por APH.

De los 103 compuestos identificados en la fracción volátil del jamón Serrano al final del periodo de refrigeración, 21 compuestos resultaron afectados significativamente por el tratamiento de APH. Doce compuestos volátiles (1-octanol, 1-penten-3-ol, 1-octen-3-ol, hexano, dodecano, 2-propanona, 2-butanona, 2-pentanona, 5-metil-3-heptanona, 3-fenil-2-propenal, metanotiol y dióxido de sulfuro)

alcanzaron mayores niveles en los jamones Serrano tratados por APH mientras que 9 compuestos (2-butanol, 2-pentanol, 2-hexanol, 2-heptanol, 3-metil-2-butanol, acetato de etilo, butanoato de etilo, sulfuro de dimetilo y disulfuro de dimetilo) alcanzaron menores niveles en los jamones Serrano tratados por APH.

En **jamón Ibérico** al principio del periodo de refrigeración, 35 de los 122 compuestos identificados en la fracción volátil resultaron afectados significativamente por el tratamiento de APH. Cuatro compuestos volátiles (nonanal, hexadecano, metanotiol y trisulfuro de dimetilo) alcanzaron mayores niveles en los jamones Ibéricos tratados por APH mientras que 31 compuestos (9 ácidos, 1 alcohol, 7 aldehídos, 3 ésteres, 3 alcanos, 6 compuestos bencénicos, y 2 compuestos azufrados) alcanzaron menores niveles en los jamones tratados por APH.

De los 116 compuestos identificados en la fracción volátil del jamón Ibérico al final del periodo de refrigeración, 34 compuestos resultaron afectados significativamente por el tratamiento de APH. Once compuestos volátiles (ácido nonanoico, 1-penten-3-ol, 2-butoxietanol, decanal, 2-butanona, 2-pentanona, 2-hexanona, metanotiol, disulfuro de carbono, trisulfuro de dimetilo y *p*-nitrofenil hexanoato) alcanzaron mayores niveles en los jamones Ibéricos tratados por APH mientras que 23 compuestos (3 ácidos, 3 alcoholes, 10 aldehídos, 4 ésteres, 1 compuesto bencénico, 1 compuesto azufrado y 1 furanona) alcanzaron menores niveles en los jamones tratados por APH.

El tratamiento del jamón curado por APH parece acelerar la formación de hidroperóxidos y las reacciones de oxidación lipídica (Andrés et al., 2004). Debido a ello se pueden producir cambios en el perfil volátil que afectan a la calidad y la aceptabilidad del producto. Este hecho podría explicar por qué algunos grupos de compuestos volátiles como los ácidos, los alcoholes, los aldehídos lineales y las cetonas, que proceden de reacciones de oxidación lipídica, presentan mayores niveles en el jamón curado después de la aplicación de APH. Además, las reacciones de oxidación parecen incrementarse proporcionalmente con el aumento de la presión aplicada (Andrés et al., 2004).

En el presente estudio, el tratamiento de APH no produjo un efecto significativo en los niveles de los compuestos procedentes de la oxidación lipídica en el jamón Serrano antes del periodo de refrigeración. Solo resultaron afectados los compuestos volátiles procedentes de reacciones enzimáticas (ésteres) y del catabolismo de aminoácidos azufrados (compuestos azufrados). Sin embargo, una vez trascurridos los 5 meses de refrigeración, se observó en el jamón Serrano tratado por APH un aumento significativo de compuestos procedentes de las reacciones de oxidación lipídica tales como algunos alcoholes y cetonas. En jamón Serrano tratado a 400 MPa y almacenado durante 3 días en refrigeración tampoco se observó un incremento de los compuestos volátiles procedentes de reacciones de oxidación lipídica (Rivas-Cañedo et al., 2009a)

En jamón Ibérico, el tratamiento de APH afectó a un número mayor de compuestos volátiles que en jamón Serrano. Los compuestos volátiles afectados procedían principalmente de reacciones de oxidación lipídica, de reacciones de Maillard y del metabolismo microbiano. Al final del periodo de refrigeración, en jamón Ibérico se observó un aumento significativo de la formación de compuestos volátiles procedentes de las reacciones de oxidación con respecto al inicio del periodo de refrigeración. En un trabajo anterior se había observado un incremento significativo de la formación de aldehídos lineales, considerados indicadores de la oxidación lipídica, y de la percepción de olor rancio en el análisis sensorial de jamón Ibérico tratado a 600 MPa y almacenado durante 30 días (Fuentes et al., 2010).

El tratamiento de APH no solo acelera la formación de hidroperóxidos y las reacciones de oxidación lipídica, sino que también puede modificar la estructura de las enzimas y de sus sustratos (Cheftel & Culioli, 1997). Esto justificaría el que algunos de los compuestos volátiles formados por reacciones enzimáticas o por la actividad de microorganismos resultasen afectados tras aplicar el tratamiento de APH a jamón Serrano y jamón Ibérico en el presente estudio. Sin embargo, el tratamiento de jamón Serrano a 400 MPa durante 10 min a 12 ºC solamente afectó a un compuesto, el

pentanoato de etilo (Rivas-Cañedo et al., 2009a). La mayor presión (600 MPa) aplicada en el presente estudio frente a los 400 MPa del trabajo citado podría ser la responsable de esta diferencia.

Cabe señalar que cualquier incremento o disminución de los niveles de los compuestos volátiles del jamón curado tras la aplicación del tratamiento de APH podría modificar el aroma del producto y su calidad sensorial. Para una mayor precisión sobre el efecto de las APH, habría que tener en consideración cuales son los compuestos volátiles de impacto sobre el aroma del jamón curado, lo que se puede determinar mediante técnicas de olfatometría.

8.4. Efecto del almacenamiento en refrigeración del jamón curado sobre los compuestos volátiles

En **jamón Serrano** se identificaron 100 compuestos volátiles al principio de la refrigeración y 103 compuestos al final de la refrigeración, con independencia de la composición química y el tratamiento por APH de las muestras. De dichos compuestos, 95 se identificaron al principio y al final de la refrigeración mientras que 5 compuestos (2-heptanol, 2-metil-2-buten-1-ol, 2,3-pentanodiona, estireno 1 y 2) se identificaron solamente al principio y 8 compuestos (ácido heptanoico, 1-propanol, 3-metil-2-butanol, decano, alcano ramificado, decanoato de etilo, 2-etilfurano y 2,3,5-trimetilpirazina) solamente al final. Al final de la refrigeración, la suma total de los niveles de compuestos volátiles fue un 32,7 % menor en las muestras control y un 20,3 % menor en las muestras tratadas por APH que al principio de la misma. Sin embargo, dichas diferencias no fueron significativas ($P > 0,05$).

Durante la refrigeración del jamón Serrano control, los niveles de alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanonas, terpenos y compuestos misceláneos disminuyeron ($P < 0,05$) mientras que en el jamón Serrano tratado por APH los niveles de alcoholes, cetonas, terpenos y compuestos misceláneos disminuyeron ($P < 0,05$) y los niveles de furanos aumentaron ($P < 0,05$) (Tabla 8.2).

Respecto a los compuestos volátiles individuales del jamón Serrano, 36 compuestos disminuyeron significativamente o desaparecieron durante la refrigeración mientras que 13

compuestos aumentaron significativamente o aparecieron durante la refrigeración (Tabla 8.3). De estos 49 compuestos volátiles, 45 compuestos resultaron afectados por la refrigeración en las muestras control, 46 compuestos en las muestras tratadas por APH y 42 compuestos tanto en las muestras control como en las muestras tratadas por APH. Además del efecto propiamente dicho del almacenamiento en refrigeración, las diferencias de composición química entre los jamones Serranos analizados al principio y al final de la refrigeración (Tabla 8.1.) deben ser tomadas en consideración, ya que estas diferencias de composición química también pueden afectar a los niveles de los compuestos volátiles del jamón Serrano.

Tabla 8.2. Efecto de la refrigeración durante 5 meses a 4 ^{o}C sobre los niveles de los grupos de compuestos volátiles en jamón Serrano control y tratado por alta presión (APH)

Grupos de volátiles[1]	Control			APH		
	0 meses	5 meses	P^2	0 meses	5 meses	P^2
Ácidos	1495.83	1655.28	NS	1353.23	1562.29	NS
Alcoholes	4093.81	3044.41	**	3967.56	2658.88	***
Aldehídos	1578.30	1020.31	***	1323.88	1100.76	NS
Cetonas	4346.92	1787.46	***	4511.01	2852.39	***
Ésteres	101.91	117.32	NS	82.22	81.06	NS
Alcanos	1418.39	944.95	NS	1023.46	1314.16	NS
Compuestos bencénicos	618.87	425.15	**	602.99	493.39	NS
Compuestos azufrados	364.71	287.67	*	220.73	196.91	NS
Furanos	144.62	164.77	NS	120.78	186.02	*
Furanonas	105.64	93.02	*	106.74	94.09	NS
Pirazinas	123.28	150.93	NS	126.19	167.13	NS
Terpenos	5.92	3.04	*	6.43	3.15	**
Otros compuestos	81.17	50.65	***	78.92	61.88	*
Total volátiles	14479.37	9744.97	***	13524.14	10772.11	**

[1] Los niveles de los grupos son la suma de los niveles de los compuestos volátiles individuales.

[2] Significación estadística según ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significativo.

Tabla 8.3. Efecto de la refrigeración durante 5 meses a 4 ºC sobre los niveles de los compuestos volátiles individuales de jamón Serrano control y tratado por alta presión (APH)

	Control			APH		
Compuestos volátiles[1]	0 meses	5 meses	P^2	0 meses	5 meses	P^2
Ácido pentanoico	324.65	488.20	*	295.26	436.28	NS
Ácido hexanoico	0.00	10.03	***	0.00	9.92	***
1-Propanol	0.00	14.40	***	0.00	12.53	***
1-Butanol	82.42	43.46	***	83.33	47.07	***
1-Pentanol	455.35	216.43	***	442.94	227.70	***
1-Hexanol	240.06	167.63	NS	210.32	161.55	*
1-Octanol	4.01	1.58	***	3.94	2.63	*
2-Propanol	579.64	343.18	***	609.30	306.33	***
2-Heptanol	49.70	0.00	***	41.84	0.00	***
3-Metil-2-butanol	0.00	10.70	***	0.00	7.17	***
2-Metil-2-buten-1-ol	11.02	0.00	***	11.03	0.00	***
2-Metil-3-buten-2-ol	17.42	6.15	*	19.37	6.65	*
2-Butoxietanol	852.14	491.59	**	864.65	507.02	**
1-Metoxi-2-propanol	82.93	2.51	***	76.23	2.70	***
Heptanal	12.79	8.97	*	12.55	7.70	*
Nonanal	19.15	50.19	***	22.40	40.44	**
Dodecanal	3.92	8.32	**	3.99	5.81	*
2-Metilbutanal	413.24	33.81	***	356.92	30.69	***
Hexano	44.41	55.83	NS	30.26	103.96	***
Decano	0.00	29.58	***	0.00	26.76	***
Undecano	49.19	68.56	NS	48.80	83.90	**
Dodecano	387.09	97.96	***	397.48	179.60	***
Alcano ramificado	0.00	18.82	***	0.00	19.15	***
3-Metilbutanoato de etilo	3.22	1.87	*	2.44	1.44	NS
Decanoato de etilo	0.00	3.42	***	0.00	3.04	***
2-Propanona	838.45	423.77	***	870.15	669.48	**
2-Butanona	614.05	341.01	***	645.02	470.41	**
2-Pentanona	1837.94	488.42	***	1901.76	994.78	**
2-Heptanona	648.43	282.77	***	662.12	420.71	*
2-Octanona	50.12	31.88	*	52.37	38.98	NS
2,3-Pentadiona	4.25	0.00	***	4.34	0.00	***
3-Hidroxi-2-butanona	331.38	199.68	**	352.20	234.75	*
4-Metil-2-pentanona	4.38	1.95	***	4.49	2.35	***
5-Metil-3-heptanona	4.79	6.89	**	5.15	8.95	***
Etilbenceno	6.18	4.46	**	6.44	4.58	**
Etil estireno 1	19.05	0.00	***	19.12	0.00	***
Etil estireno 2	16.34	0.00	***	16.44	0.00	***
Benzonitrilo	7.66	4.67	***	7.37	5.38	***
4-Metil-fenol	8.76	5.83	**	8.29	5.79	**

3-Fenil-2-propenal	5.90	1.95	***	5.86	2.31	***
4-Fenil-3-buten-2-ona	8.72	6.17	***	8.50	6.32	***
Naftaleno	6.59	4.24	***	6.22	4.17	***
Dioxido de sulfuro	6.98	5.20	***	14.92	11.00	**
2,3,5-Trimetilfurano	3.38	1.32	*	2.93	1.38	***
2,3-Dihidro-4-metilfurano	3.21	1.49	***	2.78	1.55	***
2-Etilfurano	0.00	53.88	***	0.00	55.28	***
2,3,5-Trimetilpirazina	0.00	28.55	***	0.00	34.00	***
α-Pineno	10.66	1.53	NS	4.58	1.55	**
p-Nitrofenil hexanoato	43.07	16.72	***	40.22	27.71	*

[1] Los niveles de volátiles son la suma de las abundancias de los iones característicos, multiplicada por 10^{-5}.

[2] Significación estadística según ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significativo.

En jamón Serrano, los compuestos presumiblemente procedentes de reacciones de oxidación lipídica (ácidos lineales, alcoholes lineales, aldehídos lineales, cetonas lineales e hidrocarburos), de reacciones de Maillard (ácidos ramificados, alcoholes ramificados, aldehídos ramificados, cetonas ramificadas, compuestos nitrogenados y azufrados) y de origen microbiano o desconocido (ácido acético y ésteres) representaron respectivamente el 72,7 %, 22,5 % y 4,8 % de la fracción volátil al principio de la refrigeración mientras que al final de la refrigeración representaron respectivamente el 67,4 %, 25,4 % y 7,2 % de la fracción volátil. Estas variaciones en los porcentajes de los tres grupos de compuestos se explican fundamentalmente por el fuerte descenso de los niveles totales de los compuestos volátiles derivados de la oxidación lipídica durante la refrigeración, próximo al 33 %, por un descenso algo menor de los compuestos volátiles procedentes de reacciones de Maillard, próximo al 18%, y por un ligero aumento de los compuestos volátiles de origen microbiano o desconocido, próximo al 8 %.

En **jamón Ibérico** se identificaron 122 compuestos volátiles al principio de la refrigeración y 116 compuestos al final de la misma, con independencia de la composición química y del tratamiento por APH. De ellos, 109 compuestos volátiles se identificaron tanto al principio como al final de la refrigeración mientras que 13 compuestos (1-hidroxi-2-butanona, 6-metil-2-heptanona, butanoato de etilo, 3-metilbutanoato de etilo, heptanoato de etilo, nonano, hexadecano, 1-hepteno, 2-octeno, *m*-

etiltolueno, 2,4-dimetilfenol, feniletanol y pirrol) se identificaron solamente al principio de la refrigeración y 7 compuestos (ácido nonanoico, 2-etil-1-hexanol, butanal, decanal, acetofenona, éter etílico y 2-metil-1,3-tiazol) se identificaron solamente al final. La suma total de los niveles de compuestos volátiles del jamón Ibérico fue 5,1 % menor en las muestras control y 1,3 % menor en las muestras tratadas por APH al final de la refrigeración que al principio de las misma. Estas diferencias no fueron significativas ($P > 0,05$).

Los niveles totales de ácidos, alcoholes, cetonas, ésteres, compuestos azufrados, furanos, furanonas, pirazinas y compuestos misceláneos disminuyeron ($P < 0,05$) y los niveles totales de compuestos bencénicos aumentaron ($P < 0,05$) durante la refrigeración de las muestras control de jamón Ibérico. Los niveles totales de ácidos, alcoholes, cetonas, ésteres, furanos, furanonas y pirazinas disminuyeron ($P < 0,05$) mientras que los niveles totales de compuestos bencénicos, compuestos azufrados y compuestos misceláneos aumentaron ($P < 0,05$) durante la refrigeración en las muestras tratadas por APH (Tabla 8.4).

Tabla 8.4. Efecto de la refrigeración durante 5 meses a 4 ºC sobre los grupos de compuestos volátiles del jamón Ibérico control y tratado por alta presión (APH)

	Control			APH		
Grupos de volátiles[1]	0 meses	5 meses	P^2	0 meses	5 meses	P^2
Ácidos	1540.40	1297.86	***	1378.13	1226.93	*
Alcoholes	2142.75	1856.30	*	2117.69	1738.27	**
Aldehídos	1743.93	1923.23	NS	1520.18	1708.41	NS
Cetonas	1800.20	1542.92	***	1857.17	1618.14	**
Ésteres	136.25	77.81	**	131.13	67.37	***
Alcanos	1654.10	1381.00	NS	1748.24	1397.18	NS
Compuestos bencénicos	1323.51	1960.99	***	1031.63	1970.23	***
Compuestos azufrados	413.82	287.55	***	385.86	474.14	*
Furanos	178.85	84.32	**	199.37	75.70	**
Furanonas	121.03	103.74	**	115.46	100.68	**
Pirazinas	88.44	56.73	***	91.64	57.36	***
Otros compuestos	24.67	24.52	NS	24.75	30.12	*
Total volátiles	11167.97	10596.96	NS	10601.25	10464.54	NS

[1] Los niveles de los grupos son la suma de los niveles de los compuestos volátiles individuales.

[2] Significación estadística según ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significativo.

Respecto a los compuestos volátiles individuales del jamón Ibérico, 50 compuestos disminuyeron significativamente o desaparecieron durante la refrigeración y 25 compuestos aumentaron significativamente o aparecieron durante la refrigeración (Tabla 8.5). De estos 75 compuestos, 64 compuestos resultaron afectados por la refrigeración en las muestras control, 72 compuestos en las muestras tratadas por APH y 61 compuestos tanto en las muestras control como en las muestras tratadas por APH. Al contrario de lo observado para el jamón Serrano, no existían diferencias significativas en la composición química del jamón Ibérico al principio y al final de la refrigeración, por lo que este efecto sería debido únicamente a la refrigeración.

Tabla 8.5. Efecto de la refrigeración durante 5 meses a 4 ºC sobre los compuestos volátiles individuales del jamón Ibérico control y tratado por alta presión (APH)

	Control			APH		
Compuestos volátiles[1]	0 meses	5 meses	P^2	0 meses	5 meses	P^2
Ácido hexanoico	335.53	187.86	***	304.83	175.42	***
Ácido octanoico	18.25	24.97	***	16.20	23.86	***
Ácido nonanoico	0.00	23.59	***	0.00	29.29	***
Etanol	809.91	489.06	**	824.35	469.99	**
2-Propanol	254.82	357.37	***	255.04	349.76	***
1-Metoxi-2-propanol	18.22	9.77	**	18.05	10.53	*
2-Pentanol	80.11	117.55	**	59.80	58.93	NS
1-Hexanol	283.90	218.13	**	274.44	202.11	**
1-Octanol	2.26	10.64	***	2.32	10.84	***
2-Etil-1-hexanol	0.00	59.34	***	0.00	59.02	***
Etanal	15.08	8.79	**	12.07	7.47	**
Butanal	0.00	63.68	***	0.00	55.27	***
2-Metilpropanal	74.16	96.89	***	63.92	78.82	**
Heptanal	51.68	65.59	NS	39.95	57.46	***
Octanal	12.49	26.84	***	13.89	25.17	***
Nonanal	27.65	85.93	***	34.77	93.56	***
Decanal	0.00	53.41	***	0.00	101.91	***
2-Propanona	485.49	432.07	***	494.06	445.66	***
1-Hidroxi-2-butanona	14.21	0.00	***	9.46	0.00	***
2,3-Pentanediona	5.12	2.87	***	4.39	2.79	***
6-Metil-2-heptanona	1.77	0.00	***	1.05	0.00	***
3-Octanona	9.21	4.52	NS	6.40	4.22	***
2,3-Octanediona	9.03	4.54	**	7.32	4.64	**

2-Nonanona	9.33	4.75	*	7.09	4.88	NS
Acetato de etilo	50.37	35.86	*	48.99	30.75	**
Propanoato de etilo	5.07	3.67	NS	4.49	2.98	*
2-Hidroxipropanoato de etilo	11.58	8.20	NS	10.53	7.23	*
Butanoato de etilo	11.19	0.00	***	10.19	0.00	***
3-Metilbutanoato de etilo	10.75	0.00	***	10.65	0.00	***
Hexanoato de etilo	32.46	18.89	**	32.21	16.17	**
Heptanoato de etilo	2.51	0.00	***	2.02	0.00	***
Octanoato de etilo	5.73	2.94	**	5.46	2.68	**
Hexano	101.15	47.09	*	99.52	50.09	*
Heptano	462.31	229.26	*	499.95	234.49	**
1-Hepteno	6.29	0.00	***	7.03	0.00	***
Octano	858.85	503.62	NS	932.72	490.69	*
2-Octeno	4.00	0.00	***	4.29	0.00	***
Nonano	5.35	0.00	***	6.06	0.00	***
Hexadecano	1.51	0.00	***	1.63	0.00	***
Ciclohexano	62.15	401.68	***	41.32	417.30	***
Alcano ramificado I	13.61	23.52	***	9.26	22.29	***
Alcano ramificado II	10.35	19.57	***	7.21	18.67	***
Etilbenceno	128.75	284.27	***	88.83	294.95	***
1-Metiletil-benceno	5.22	58.30	***	3.51	59.99	***
Trimetilbenceno	14.05	5.86	NS	8.78	6.35	**
o-Etiltolueno	2.48	3.91	***	2.06	4.24	***
Estireno	560.11	1150.69	***	342.03	1141.31	***
Benzonitrilo	1.80	1.11	*	2.10	1.29	NS
Fenol	6.39	3.57	NS	4.68	3.47	***
4-Metil-fenol	3.42	2.16	NS	2.87	2.10	***
2,4-Dimetilfenol	3.46	0.00	***	1.76	0.00	***
4-Etilfenol	33.98	24.41	***	34.57	24.18	***
4-Pentilfenol	2.99	2.01	**	2.78	2.06	**
p-Xileno	9.00	12.17	NS	9.27	15.12	*
Fenil-metanol	13.84	0.00	***	5.85	0.00	***
2-Fenil-etanol	159.87	109.93	***	172.64	115.21	***
Feniletanal	94.61	58.00	**	82.09	50.30	**
1-Fenil-propano	21.52	10.24	***	12.95	10.59	*
Naftaleno	6.69	3.66	**	5.26	3.94	***
Acetofenona	0.00	21.02	***	0.00	21.36	***
Metanotiol	16.21	9.14	***	32.21	15.57	***
Disulfuro de carbono	226.08	39.86	***	207.57	45.51	***
Sulfuro de dimetilo	7.41	5.69	NS	4.55	3.23	**
Disulfuro de dimetilo	135.29	186.83	**	88.72	205.97	***
Trisulfuro de dimetilo	28.84	46.03	**	52.82	203.86	***
2-Butilfurano	20.88	11.07	**	21.61	10.32	**
2-Pentilfurano	113.20	63.10	NS	131.00	55.69	*
2(3H)-5-Metildihidrofuranona	10.93	6.84	*	8.46	6.76	***
2(3H)-5-Butildihidrofuranona	7.02	4.74	***	6.78	4.57	***

Metilpirazina	10.63	7.10	***	10.77	6.96	***
Dimetilpirazina	66.57	43.88	***	69.13	44.61	***
2,3,5-Trimetilpirazina	11.24	5.75	*	11.74	5.79	*
Pirrol	2.35	0.00	***	1.74	0.00	***
Éter etílico	0.00	11.71	***	0.00	10.83	***
2-Metiltiazol	0.00	18.79	***	0.00	19.24	***

[1] Los niveles de volátiles son la suma de las abundancias de los iones característicos, multiplicada por 10^{-5}.

[2] Significación estadística según ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no significativo.

Como ya se ha comentado, el mayor contenido de grasa intramuscular del jamón Ibérico en comparación con el jamón Serrano podría ser, al menos en parte, responsable del mayor número de compuestos identificados en su fracción volátil, debido a la relación existente entre la grasa intramuscular y los fenómenos de oxidación lipídica (Fuentes et al., 2014a, b). Por otra parte, se ha comprobado que los materiales plásticos de envasado pueden contribuir a la fracción volátil durante el almacenamiento del jamón en refrigeración, por fenómenos de migración de algunos hidrocarburos y compuestos bencénicos (Rivas-Cañedo et al., 2009a, b).

En las muestras control de jamón Ibérico analizadas al principio de la refrigeración los compuestos presumiblemente procedentes de reacciones de oxidación lipídica (ácidos lineales, alcoholes lineales, aldehídos lineales, cetonas lineales e hidrocarburos), de reacciones de Maillard (ácidos ramificados, alcoholes ramificados, aldehídos ramificados, cetonas ramificadas, compuestos nitrogenados y azufrados) y de origen microbiano o desconocido (ácido acético y ésteres) representaron el 75,0 %, 18,1 % y 6,9 % respectivamente de la fracción volátil mientras que al final de la refrigeración representaron respectivamente el 75,1 %, 18,7 % y 6,2 %. En contraste con lo registrado para el jamón Serrano, las variaciones en los porcentajes de estos tres grupos de compuestos volátiles fueron mínimas durante la refrigeración del jamón Ibérico, atribuibles a unos balances similares de las reacciones de formación y degradación de los volátiles originados por estas tres vías. Esto puede deberse al mayor contenido en grasa intramuscular del jamón Ibérico y, posiblemente también, a unos periodos de elaboración y maduración más prolongados que dan lugar a un producto

más estable. En estudios previos sobre jamón Ibérico no refrigerado los porcentajes de los compuestos volátiles originados por estas tres vías fueron del 81,6 %, 12,7 % y 5,7 %, respectivamente (Ramírez & Cava, 2007).

8.5. Efecto de la composición química sobre la microbiota del jamón curado

El **contenido en grasa intramuscular del jamón Serrano** influyó sobre los niveles de algunos de los grupos microbianos. Al principio del periodo de refrigeración, el contenido de grasa intramuscular de los jamones control afectó significativamente a los niveles de aerobios mesófilos, psicrotrofos y mohos y levaduras mientras que en los jamones tratados por APH la grasa no afectó significativamente a los niveles de ningún grupo microbiano. Los jamones control con bajo contenido en grasa intramuscular mostraron por lo general niveles más elevados de microorganismos. Al final del periodo de refrigeración, el contenido de grasa intramuscular influyó sobre la microbiota tanto de los jamones control como de los jamones tratados por APH, afectando significativamente a los niveles de aerobios mesófilos, psicrotrofos, micrococáceas y mohos y levaduras. El jamón Serrano con bajo contenido de grasa intramuscular mostró por lo general niveles más elevados de microorganismos, al igual que ocurría al principio de la refrigeración.

El **contenido en grasa intramuscular del jamón Ibérico** no influyó significativamente sobre ninguno de los grupos microbianos al principio del periodo de refrigeración. Al final de la refrigeración, el contenido de grasa intramuscular no afectó significativamente a los niveles de ningún grupo microbiano en los jamones control mientras que en los jamones tratados por APH afectó significativamente a los niveles de mohos y levaduras.

La sal añadida durante el proceso de elaboración del jamón curado cumple distintas funciones entre las cuales destaca la inhibición del crecimiento microbiano a través de la reducción de la a_w, la solubilización de las proteínas y la regulación de las reacciones de proteolisis y lipolisis, lo que afecta a la textura y el flavor del producto (Toldrá & Flores, 1998). La sal es la principal fuente de

estafilococos y micrococos y ejerce una presión selectiva sobre el tipo y los niveles de otros microorganismos en el jamón curado (Cordero & Zumalacárregui, 2000). La mayor prevalencia de los estafilococos es debida precisamente a su resistencia a altas concentraciones de sal y a un bajo potencial redox (Lorenzo et al., 2012).

La **concentración de sal del jamón Serrano** influyó considerablemente sobre los niveles de los principales grupos microbianos. Al principio del periodo de refrigeración, la concentración de sal de los jamones control afectó a los niveles de aerobios mesófilos, psicrotrofos, bacterias lácticas y mohos y levaduras mientras que en los jamones tratados por APH la concentración de sal no afectó a los niveles de ningún grupo microbiano. Los jamones con elevada concentración de sal mostraron mayores niveles de aerobios mesófilos, psicrotrofos y mohos y levaduras y los de concentraciones intermedias de sal mayores niveles de bacterias lácticas. Al final del periodo de refrigeración, la concentración de sal de los jamones Serrano no afectó significativamente a los niveles de ningún grupo microbiano ni en los jamones control ni en los tratados por APH.

La **concentración de sal del jamón Ibérico**, no influyó significativamente sobre ninguno de los grupos microbianos estudiados en las muestras control y en las muestras tratadas por APH, ni al principio de la refrigeración ni al final de la misma.

La **relación sal en magro del jamón Serrano** influyó de manera similar a la concentración de sal sobre los microorganismos. Al principio del periodo de refrigeración, la relación sal en magro de los jamones control afectó a los niveles de aerobios mesófilos, psicrotrofos y mohos y levaduras mientras que en los jamones tratados por APH la relación sal en magro no afectó significativamente a los niveles de ningún grupo microbiano. Los jamones con elevada relación sal en magro mostraron mayores niveles de aerobios mesófilos, psicrotrofos y mohos y levaduras. Al final del periodo de refrigeración, la relación sal en magro de los jamones Serranos tanto controles como tratados por APH no afectó significativamente a los niveles de ningún grupo microbiano.

La **relación sal en magro del jamón Ibérico** apenas influyó sobre los microorganismos del mismo. Al principio del periodo de refrigeración, la relación sal en magro de los jamones control no afectó significativamente a ningún grupo microbiano mientras que en los jamones tratados por APH la relación sal en magro afectó únicamente a los niveles de bacterias lácticas. Los jamones con baja relación sal en magro mostraron los mayores niveles de bacterias lácticas. Al final del periodo de refrigeración, la relación sal en magro de los jamones Ibéricos tanto controles como tratados por APH no afectó significativamente a los niveles de ninguno de los grupos microbianos estudiados.

Los valores elevados de a_w favorecen generalmente el crecimiento y el metabolismo de los microorganismos. Sin embargo, algunas especies xerófilas de mohos y levaduras son capaces de crecer en carnes y productos cárnicos con valores de a_w bajos (Rubio et al., 2007a). La resistencia de los microrganismos al tratamiento de APH aumenta conforme disminuyen los valores de a_w como muestras un estudio de inactivación de S. *aureus* y bacterias lácticas después de un tratamiento de APH de 600 MPa durante 6 min en alimentos tales como carne marinada, jamón cocido y jamón curado con diferentes valores de a_w (Hugas et al., 2002). Cuando se comparó la resistencia al tratamiento de APH de *L. monocytogenes* en jamón Serrano y jamón Ibérico, se observó que los valores más bajos de a_w del jamón Serrano parecían ejercer un efecto protector durante el tratamiento de APH, con niveles más elevados del patógeno inmediatamente después del tratamiento, aunque durante la semana posterior al tratamiento el descenso de los niveles de *L. monocytogenes* fue más acusado en jamón Serrano (Morales et al., 2006).

La **actividad de agua del jamón Serrano** apenas influyó sobre los niveles de microorganismos. Al principio del periodo de refrigeración, la a_w de los jamones tanto controles como tratados por APH no afectó significativamente a los niveles de ninguno de los grupos microbianos. Este resultado parece contradecir el hecho de que algunos compuestos volátiles de origen presumiblemente microbiano mostraran mayores niveles en los jamones con valores altos de a_w. Al final de periodo de refrigeración, la a_w de los jamones control influyó en los niveles de micrococáceas mientras que la a_w de los jamones

tratados por APH afectó a los niveles de aerobios mesófilos, psicrotrofos y micrococáceas. Los jamones con valores intermedios de a_w fueron los que mostraron los mayores niveles de aerobios mesófilos, psicrotrofos y micrococáceas.

La **actividad de agua del jamón Ibérico** tuvo un efecto limitado sobre los niveles de microorganismos. Al principio del periodo de refrigeración, la a_w de los jamones control afectó únicamente a los niveles de psicrotrofos y micrococáceas mientras que en los jamones tratados por APH no afectó significativamente a ningún grupo microbiano. Los jamones con valores elevados de a_w mostraron mayores niveles de psicrotrofos y micrococáceas. Al final del periodo de refrigeración, la a_w de los jamones Ibéricos tanto controles como tratados por APH no afectó significativamente a los niveles de ningún grupo microbiano.

De acuerdo con los resultados obtenidos, la influencia de las variables de composición (contenido de grasa intramuscular, concentración de sal, relación sal en magro y a_w) sobre los niveles de microorganismos fue mayor en jamón Serrano que en jamón Ibérico. En jamón Serrano, la variable de composición que más influyó sobre los niveles de microorganismos fue la concentración de sal, que afectó significativamente a los niveles de aerobios mesófilos, psicrotrofos, bacterias lácticas y mohos y levaduras en las muestras control. Por el contrario, la variable de composición que más influyó sobre los niveles de microorganismos en jamón Ibérico fue la a_w, que afectó significativamente a los niveles de psicrotrofos y micrococáceas en las muestras control.

8.6. Efecto del tratamiento por altas presiones y el almacenamiento en refrigeración sobre la microbiota del jamón curado

El **tratamiento por altas presiones** redujo significativamente los niveles de todos los grupos microbianos estudiados tanto en jamón Serrano como en jamón Ibérico. En **jamón Serrano**, el tratamiento de APH redujo los aerobios mesófilos en 1,63 log ufc g^{-1}, los psicrotrofos en 1,71 log ufc g^{-1}, las bacterias lácticas en 0,44 log ufc g^{-1}, las micrococáceas en 1,15 log ufc g^{-1} y los mohos y

levaduras en 1,95 log ufc g^{-1}. En **jamón Ibérico** el tratamiento de APH redujo los aerobios mesófilos en 1,78 log ufc g^{-1}, los psicrotrofos en 2,00 log ufc g^{-1}, las bacterias lácticas en 2,04 log ufc g^{-1}, las micrococáceas en 1,74 log ufc g^{-1} y los mohos y levaduras en 1,87 log ufc g^{-1}.

Durante el **almacenamiento en refrigeración**, los niveles de microorganismos aumentaron en el **jamón Serrano control**, con incrementos de 0,66 log ufc g^{-1} para aerobios mesófilos, 0,51 log ufc g^{-1} para bacterias lácticas, 0,85 log ufc g^{-1} para psicrotrofos, 0.31 log ufc g^{-1} para micrococáceas y 0.68 log ufc g^{-1} para mohos y levaduras.

En el **jamón Ibérico control**, los niveles de microorganismos apenas variaron durante el almacenamiento en refrigeración, con un incremento de 0,39 log ufc g^{-1} para bacterias lácticas y descensos de 0,36 log ufc g^{-1} para aerobios mesófilos, 0,04 log ufc g^{-1} para psicrotrofos, 0,28 log ufc g^{-1} para micrococáceas y 0,31 log ufc g^{-1} para mohos y levaduras.

Durante el almacenamiento en refrigeración del **jamón Serrano tratado por APH** se observó una recuperación de la mayoría de los grupos microbianos, con incrementos de 2,30 log ufc g^{-1} para aerobios mesófilos, 2,47 log ufc g^{-1} para psicrotrofos, 1,82 log ufc g^{-1} para micrococáceas y 2,30 log ufc g^{-1} para mohos y levaduras. Al final de la refrigeración se llegaron a alcanzar niveles muy próximos a los de las muestras control, con excepción de las bacterias lácticas que se mantuvieron en niveles 1,02 log ufc g^{-1} inferiores a los de las muestras control.

En **jamón Ibérico tratado por APH** los niveles de los grupos microbianos aumentaron solo ligeramente durante la refrigeración, con incrementos de 0,89 log ufc g^{-1} para aerobios mesófilos, 1,09 log ufc g^{-1} para bacterias lácticas, 0,94 log ufc g^{-1} para psicrotrofos, 0,51 log ufc g^{-1} para micrococáceas y 0,74 log ufc g^{-1} para mohos y levaduras. Como consecuencia de ello, al final del periodo de refrigeración los niveles en las muestras tratadas por APH siguieron siendo inferiores a los niveles de las muestras control, con diferencias que oscilaban entre 0,53 y 1,34 log ufc g^{-1} para los diferentes grupos microbianos.

Por lo que respecta a la **biodiversidad de la microbiota**, la aplicación de la técnica de DGGE a las muestras de **jamón Serrano** al inicio de la refrigeración, a pesar de sus limitaciones debido a los reducidos niveles de microorganismos presentes en el producto, permitió poner de manifiesto que la microbiota del jamón Serrano estaba compuesta principalmente por las especies bacterianas *S. equorum, S. succinus* y *B. subtilis*, y por las especies eucariotas *P. commune, P. chrysogenum, A. fumigatus, S. sclerotiorum, E. athecium, M. mellis, D. hansenii* y *C. glucosophila*. El tratamiento del jamón Serrano por APH produjo ligeras variaciones en la población microbiana determinada por técnicas moleculares, tales como la desaparición de las bandas de *B. subtilis* y la atenuación de las bandas de *E. athecium*.

La microbiota del **jamón Ibérico** (Tabla 8.6), estudiada mediante identificación molecular de 227 aislados bacterianos y 24 aislados de levaduras, estaba integrada mayoritariamente por bacterias del género *Staphylococcus* y por levaduras del género *Debaryomyces*, que constituyeron un 96,0 % y 75,0 % del número total de aislados, respectivamente. La especie bacteriana más abundante fue *S. equorum*/*lugdunensis* (63,4 % del número total de aislados bacterianos), seguida de *S. epidermidis* (17,2 %) y *S. pasteuri*/*warneri* (11,0 %). Algunos de los aislados bacterianos pertenecían a géneros como *Tetragenococcus* y *Carnobacterium* que no habían sido detectados con anterioridad en jamón curado. Entre las levaduras, *Debaryomyces hansenii* fue la especie predominante.

Tabla 8.6. Identificación a nivel de género y especie de 227 aislados bacterianos y 24 aislados de levaduras de jamón Ibérico control (C) y tratado por altas presiones (APH) después de 0 y 5 meses en refrigeración.

Jamón	Tiempo (meses)	Género	Especie	Número
C	0	*Staphylococcus*	*equorum/lugdunensis*	38
			epidermidis	13
			pasteuri/warneri	8
			saprophyticus	1
		Debaryomyces	*hansenii*	2
		Moniliella	*nigrescens*	2
	5	*Staphylococcus*	*equorum/lugdunensis*	46
			epidermidis	3
			hominis	2
			pasteuri/warneri	4
		Kocuria	*palustris*	2
		Tetragenococcus	*halophilus*	1
			koreensis	1
			solitarius	2
		Debaryomyces	*hansenii*	5
APH	0	*Staphylococcus*	*equorum/lugdunensis*	12
			epidermidis	23
			hominis	1
			pasteuri/warneri	13
		Enterococcus	*faecalis*	1
			faecium/hirae	2
		Carnobacterium	*divergens*	1
		Debaryomyces	*hansenii*	3
		Cryptococcus	*magnus*	1
	5	*Staphylococcus*	*equorum/lugdunensis*	48
		Kocuria	*rhizophila*	1
		Brachybacterium	*conglomeratum*	2
		Streptomyces	*flavofungini/lomodensis*	2
		Debaryomyces	*hansenii*	8
		Rhodotorula	*mucilaginosa*	2
		Ustilago	*cynodontis/sparsa*	1

9. Conclusiones

Primera. El contenido de grasa intramuscular fue el parámetro composicional con mayor influencia sobre la fracción volátil del jamón Serrano a los 3 días en refrigeración. En las muestras control afectó a 37 de los 100 compuestos volátiles detectados frente a 28 compuestos afectados por la concentración de sal, 27 por la relación sal en magro y 19 por la actividad de agua.

Segunda. El contenido de grasa intramuscular fue igualmente el parámetro composicional con mayor influencia sobre la fracción volátil del jamón Serrano después de 5 meses en refrigeración. En las muestras control afectó a 38 de los 103 compuestos volátiles detectados frente a solamente 4 compuestos afectados por la concentración de sal, 5 por la relación sal en magro y 4 por la actividad de agua.

Tercera. El efecto del tratamiento de APH sobre la fracción volátil del jamón Serrano fue más acusado al final que al principio del periodo de refrigeración, con 21 compuestos volátiles afectados después de 5 meses en refrigeración, de los cuales 9 disminuyeron y 12 aumentaron con el tratamiento, frente a 8 compuestos afectados a los 3 días de refrigeración, de los cuales 6 disminuyeron y 2 aumentaron con el tratamiento.

Cuarta. El almacenamiento en refrigeración durante 5 meses a 4 ºC influyó sobre la fracción volátil del jamón Serrano, con 36 compuestos volátiles que disminuyeron o desaparecieron y 13 compuestos volátiles que aumentaron o aparecieron durante este periodo. Los niveles totales de compuestos volátiles disminuyeron un 32,7 % en las muestras control y un 20,3 % en las muestras tratadas por APH durante la refrigeración.

Quinta. Los niveles de la mayoría de los grupos microbianos presentes en el jamón Serrano variaron con el contenido de grasa intramuscular, la concentración de sal y la relación sal en magro, con la excepción de las micrococáceas. El tratamiento de APH redujo significativamente los niveles de todos los grupos microbianos observándose una recuperación de dichos niveles al cabo de los 5 meses en refrigeración, con la excepción de las bacterias lácticas.

Sexta. La microbiota del jamón Serrano estaba compuesta por las especies bacterianas *Staphylococcus equorum*, *S. succinus* y *Bacillus subtilis*, así como por las especies eucariotas *Penicillium commune*, *P. chrysogenum*, *Aspergillus fumigatus*, *Sclerotinia sclerotiorum*, *Eurotium athecium*, *Moniliella mellis*, *Debaryomyces hansenii* y *Candida glucosophila*. El tratamiento de APH produjo cambios en las bandas de DGGE correspondientes a algunas especies de microorganismos.

Séptima. El contenido de grasa intramuscular fue el parámetro composicional con mayor influencia sobre la fracción volátil del jamón Ibérico a los 3 días en refrigeración, aunque su influencia fue menor que en el jamón Serrano. En las muestras control de jamón Ibérico afectó a 14 de los 122 compuestos volátiles detectados frente a solamente 2 compuestos afectados por la concentración de sal, 0 por la relación sal en magro y 2 por la actividad de agua.

Octava. La influencia de la composición química sobre la fracción volátil del jamón Ibérico después de 5 meses en refrigeración fue considerablemente menor que sobre la del jamón Serrano. En las muestras control de jamón Ibérico el contenido de grasa intramuscular afectó a 4 de los 116 compuestos volátiles detectados, la concentración de sal a 4 compuestos, la relación sal en magro a 2 y la actividad de agua a 2.

Novena. El efecto del tratamiento de APH sobre la fracción volátil del jamón Ibérico fue similar al principio y al final del periodo de refrigeración, con 35 compuestos volátiles afectados a los 3 días de refrigeración, de los cuales 31 disminuyeron y 4 aumentaron con el tratamiento, y 34 compuestos afectados después de 5 meses en refrigeración, de los cuales 23 disminuyeron y 11 aumentaron con el tratamiento.

Décima. El almacenamiento en refrigeración durante 5 meses a 4 ºC influyó sobre la fracción volátil del jamón Ibérico en mayor grado que sobre la del jamón Serrano, con 50 compuestos volátiles que disminuyeron o desaparecieron y 25 compuestos volátiles que aumentaron o aparecieron durante este periodo. Los niveles totales de compuestos volátiles disminuyeron un 5,1 % en las muestras control y un 1,3 % en las muestras tratadas por APH durante este periodo, diferencias que no resultaron significativas.

Undécima. Apenas se registraron diferencias en los niveles de los grupos microbianos estudiados en el jamón Ibérico en relación con su composición química. El tratamiento de APH redujo significativamente los niveles de todos los grupos microbianos, con una recuperación de los microorganismos al cabo de los 5 meses en refrigeración inferior a la registrada en el jamón Serrano.

Duodécima. La microbiota del jamón Ibérico estaba compuesta mayoritariamente por bacterias pertenecientes al género *Staphylococcus*, y levaduras del género *Debaryomyces*. Las especies predominantes fueron *Staphylococcus equorum* y *Debaryomyces hansenii*. También se detectó la presencia de aislados pertenecientes a los géneros *Tetragenococcus* y *Carnobacterium*, que no habían sido encontrados anteriormente en jamón curado.

10. Bibliografía

Altschul, S. F., Madden, T. L., Schaeffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389-3402.

Amadoro, C., Rossi, F., Piccirilli, M., & Colavita, G. (2015). *Tetragenococcus koreensis* is part of the microbiota in a traditional Italian raw fermented sausage. *Food Microbiology*, 50, 78-82.

Andersen, H. J., & Hinrichsen, L. L. (1995). Changes in curing agents, microbial counts and volatile compounds during processing of green bacon using two different production technologies. *Journal of the Science of Food and Agriculture*, 68, 477-487.

Andrade, M. J., Córdoba, J. J., Sánchez, B., Casado, E. M., Rodríguez, M. (2009). Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. *Food Chemistry*, 113, 457-463.

Andrés, A. I., Adamsen, C. E., Møller, J. K. S., Ruiz, J., & Skibsted, L. H. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research and Technology*, 222, 486-491.

Andrés, A. I., Cava, R., Martin, D., Ventanas, J., & Ruiz, J. (2005). Lipolysis in dry-cured ham: Influence of salt content and processing conditions. *Food Chemistry*, 90, 523-533.

Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2004a). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, 84, 375-381.

Andrés, A. I., Cava, R., Ventanas, S., Muriel, E., & Ruiz, J. (2007). Effect of salt content and processing conditions on volatile compounds formation throughout the ripening of Iberian ham. *European Food Research and Technology*, 225, 677-684.

Andrés, A. I., Cava, R., Ventanas, S., Thovar, V., & Ruiz, J. (2004b). Sensory characteristics of Iberian ham: Influence of salt content and processing conditions. *Meat Science*, 68, 45-51.

Andrés, A. I., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2004c). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology*, 219, 205-210.

Antequera, M. T., & Martín, L. (2001). Reacciones químicas y bioquímicas que se desarrollan durante la maduración del jamón Ibérico. En: Tecnología del jamón Ibérico. De los sistemas tradicionales a la explotación racional del sabor y el aroma, pp. 297-322. Editor: J. Ventanas Barroso. Editorial Mundi-Prensa, Madrid.

Armenteros, M., Aristoy, M. C., Barat, J. M., & Toldrá, F. (2009). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry*, 117, 627-633.

Armenteros, M., Toldrá, F., Aristoy, M. C., Ventanas, J., & Estévez, M. (2012). Effect of the partial replacement of sodium chloride by other salts on the formation of volatile compounds during ripening of dry-cured ham. *Journal of Agricultural and Food Chemistry*, 60, 7607-7615.

Arnoldi, A., Arnoldi, C., Baldi, O., & Griffini, A. (1987). Strecker degradation of leucine and valine in a lipidic model system. *Journal of Agricultural and Food Chemistry*, 35, 1035-1038.

AOAC (2000). *Official methods of analysis* (17th edition). Association of Official Analytical Chemists, Gaithersburg, Maryland (EE. UU.).

Aymerich, T., Jofré, A., Garriga, M., & Hugas, M. (2005). Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. *Journal of Food Protection*, 68, 173-177.

Aymerich, T., Picouet, P. A., & Monfort, J. M. (2008). Decontamination technologies for meat products. *Meat Science*, 78, 114-129.

Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, 92, 280-289.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., & Mangia, A. (1992). Flavor compounds of dry-cured ham. *Journal of Agricultural and Food Chemistry*, 40, 2389-2394.

Belletti, N., Garriga, M., Aymerich, T., & Bover-Cid, S. (2013). High pressure inactivation of a virulent *Enterococcus faecalis* on dry-cured ham: Modeling the effect of processing parameters. *Innovative Food Science and Emerging Technologies*, 18, 43-47.

Beltrán, E., Pla, R., Yuste, J., & Mor-Mur, M. (2003). Lipid oxidation of pressurized and cooked chicken: role of sodium chloride and mechanical processing on TBARS and hexanal values. *Meat Science*, 64, 19-25.

Beltrán, E., Pla, R., Capellas, M., Yuste, J., & Mor-Mur, M. (2004). Lipid oxidation and colour in pressure-and heat-treated minced chicken thighs. *Journal of the Science of Food and Agriculture*, 84, 1285-1289.

Berdagué, J. L., Bonnaud, N., Rousset, S., & Touraille, C. (1993). Influence of pig crossbreed on the composition, volatile compound content and flavor of dry cured ham. *Meat Science*, 34, 119-129.

Berdagué, J. L., Denoyer, C., Le Quéré, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agricultural and Food Chemistry*, 39, 1257-1261.

Berger, C., Khan, J. A., Molimard, P., Martin, N., & Spinnler, H. E. (1999). Production of sulfur flavors by ten strains of *Geotrichum candidum*. *Applied and Environmental Microbiology*, 65, 5510-5514.

Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R., & Parente, E. (2004). Diversity and dynamics of communities of coagulase-negative *Staphylococci* in traditional fermented sausages. *Journal of Applied Microbiology*, 97, 271-284.

Blesa, E., Aliño, M., Barat, J. M., Grau, R., Toldrá, F., & Pagán, M. J. (2008). Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, 78, 135-142.

Bover-Cid, S., Belletti, N., Aymerich, T., & Garriga, M. (2015). Modeling the protective effect of a_w and fat content on the high pressure resistance of *Listeria monocytogenes* in dry-cured ham. *Food Research International*, 75, 194-199.

Bover-Cid, S., Belletti, N., Aymerich, T., & Garriga, M. (2017). Modelling the impact of water activity and fat content of dry-cured ham on the reduction of *Salmonella enterica* by high pressure processing. *Meat Science*, 123, 120-125.

Brown, J. M., Steigerwalt, A. G., Morey, R. E., Daneshvar, M. I., Romero, L. J., & McNeil, M. M. (2006). Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp nov. and emended description of the genus *Cellulosimicrobium*. *International Journal Systematic and Evolutionary Microbiology*, 56, 801-804.

Buscailhon, S., Berdagué, J. L., & Monin, G. (1993). Time-related changes in volatile compounds of lean tissue during processing of French dry-cured ham. *Journal of the Science of Food and Agriculture*, 63, 69-75.

Campos, G., Robles, L., Alonso, R., Nuñez, M., & Picon, A. (2011). Microbial dynamics during the ripening of a mixed cow and goat milk cheese manufactured using frozen goat milk curd. *Journal of Dairy Science*, 94, 4766-4776.

Campus, M., Flores, M., Martínez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, 80, 1174-1181.

Candogan, K., Wardlaw, F.B., & Acton, J.C. (2009). Effect of starter culture on proteolytic changes during processing of fermented beef sausages. *Food Chemistry*, 116, 731-737.

Cardello, A. V., Schutz, H. G., & Lesher, L. L. (2007). Consumer perceptions of foods processed by innovative and emerging technologies: A conjoint analytic study. *Innovative Food Science and Emerging Technologies*, 8, 73-83.

Carlez, A., Rosec, J. P., Richard, N., & Cheftel, J. C. (1993). High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle. *LWT-Food Science and Technology*, 26, 357-363.

Carrapiso, A. I., & García, C. (2004). Iberian ham headspace: Odorants of intermuscular fat and differences with lean. *Journal of the Science of Food and Agriculture*, 84, 2047-2051.

Carrapiso, A. I., Martín, L., Jurado, A., & García, C. (2010). Characterization of the most odour-active compounds of bone tainted dry-cured Iberian ham. *Meat Science*, 85, 54-58.

Carrapiso, A. I., Noseda, B., García, C., Reina, R., Sánchez del Pulgar, J., & Devlieghere, F. (2015). SIFT-MS analysis of Iberian hams from pigs reared under different conditions. *Meat Science*, 104, 8-13.

Carrapiso, A. I., Ventanas, J., & García, C. (2002). Characterization of the most odor-active compounds of Iberian ham headspace. *Journal of Agricultural and Food Chemistry*, 50, 1996-2000.

Carrascosa, A. V., & Cornejo, I. (1991). Characterization of *Micrococcaceae* strains selected as potential starter cultures to Spanish dry-cured ham processes. II: slow process. *Fleischwirtschaft*, 71, 1187-1188.

Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, M. R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science and Emerging Technologies*, 10, 76-81.

Cheah, P. B., & Ledward, D. A. (1996). High pressure effects on lipid oxidation in minced pork. *Meat Science*, 43, 123-134.

Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Revista de Agroquímica y Tecnología de Alimentos*, 1, 75-90.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: a review. *Meat Science*, 46, 211-236.

Chen, H. (2007). Temperature-assisted pressure inactivation of *Listeria monocytogenes* in turkey breast meat. *International Journal of Food Microbiology*, 117, 55-60.

Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, A., & García-Regueiro, J. A. (2011). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Science and Emerging Technologies*, 12, 456-465.

Clariana, M., Guerrero, L., Sárraga, C., & Garcia-Regueiro, J. A. (2012). Effects of high pressure application (400 and 900 MPa) and refrigerated storage time on the oxidative stability of sliced skin vacuum packed dry-cured ham. *Meat Science*, 50, 323-329.

Cocolin, L., Bisson, L. F., & Mills, D. A. (2000). Direct profiling of the dynamics in wine fermentations. FEMS Microbiology Letters, 189, 81-87.

Collins, M. D., Williams, A. M., & Wallbanks, S. (1990). The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. *FEMS Microbiology Letters*, 70, 255-262.

Cordero, M. R., & Zumalacárregui, J. M. (2000). Characterization of *Micrococcaceae* isolated from salt used for Spanish dry-cured ham. *Letters in Applied Microbiology*, 31, 303-306.

Córdoba, J. J., Antequera, T., García, C., Ventanas, J., López Bote, C., & Asensio, M. A. (1994). Evolution of free amino acids and amines during ripening of Iberian cured ham. *Journal of Agricultural and Food Chemistry*, 42, 2296-2301.

Córdoba, J. J., Antequera, T., Ventanas, J., López-Bote, C., García, C., & Asensio, M. A. (1994). Hydrolysis and loss of extractability of proteins during ripening of Iberian ham. *Meat Science*, 37, 217-227.

Cornejo, I., Carrascosa, A. V., Marin, M. E., & Martin, P. J. (1992). Dry-cured ham. Considerations about the origin of microorganisms that grow on the deep muscular tissues of dry cured Spanish hams during processing. *Fleischwirtschaft*, 72, 1422-1425.

Coutron-Gambotti, C., Gandemer, G., Rousset, S., Maestrini, O., & Casabianca, F. (1999). Reducing salt content of dry-cured ham: Effect on lipid composition and sensory attributes. *Food Chemistry*, 64, 13-19.

de Alba, M., Bravo, D., & Medina, M. (2013). Inactivation of *Escherichia coli* O157: H7 in dry-cured ham by high-pressure treatments combined with biopreservatives. *Food Control*, 31, 508-513.

de Alba, M., Bravo, D., & Medina, M. (2015). Inactivation of *Listeria monocytogenes* and *Salmonella* Enteritidis in dry-cured ham by combined treatments of high pressure and the lactoperoxidase system or lactoferrin. *Innovative Food Science and Emerging Technologies*, 31, 54-59.

de Alba, M., Montiel, R., Bravo, D., Gaya, P., & Medina, M. (2012). High pressure treatments on the inactivation of *Salmonella enteritidis* and the physicochemical, rheological and color characteristics of sliced vacuum-packaged dry-cured ham. *Meat Science*, 91, 173-178.

del Olmo, A., Calzada, J., Gaya, P., & Nuñez, M. (2015). Proteolysis and flavor characteristics of Serrano ham processed under different ripening temperature conditions. *Journal of Food Science*, 80, C2404-C2412.

del Olmo, A., Calzada, J., & Nuñez, M. (2013). Lipolysis, lipid peroxidation, and color characteristics of Serrano hams from Duroc and Large White pigs during dry-curing. *Journal of Food Science*, 78, C1659-C1664.

Dias, B., & Weimer, B. (1998). Conversion of methionine to thiols by *lactococci*, *lactobacilli*, and *brevibacteria*. *Applied and Environmental Microbiology*, 64, 3320-3326.

Diez, A. M., Urso, R., Rantsiou, K., Jaime, I., Rovira, J., & Cocolin, L. (2008). Spoilage of blood sausages morcilla de Burgos treated with high hydrostatic pressure. *International Journal of Food Microbiology*, 123, 246-253.

Djaballah, H., & Rivett, A. J. (1992). Peptidylglutamyl-peptide hydrolase activity of the multicatalytic proteinase complex: evidence for a new high-affinity site, analysis of cooperative kinetics, and the effect of manganese ions. *Biochemistry*, 31, 4133-4141.

Dumont, J. P., & Adda, J. (1972). Isolement des constituants de l'arôme des fromages: comparaison de methods. *Lait*, 52, 311-323.

Duong, L. M., Jeewon, R., Lumyong, S., & Hyde, K. D. (2006). DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. *Fungal Diversity*, 23, 121-138.

Earnshaw, R. G., Appleyard, J., & Hurst, R. M. (1995). Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. *International Journal of Food Microbiology*, 28, 197-219.

Estévez, M., Ollilainen, V., & Heinonen, M. (2009). Analysis of protein oxidation markers α-aminoadipic and γ-glutamic semialdehydes in food proteins using liquid chromatography (LC)-electrospray ionization (ESI)-multistage tandem mass spectrometry (MS). *Journal of Agricultural and Food Chemistry*, 57, 3901-3910.

Facklam, R. R., & Collins, M. D. (1989). Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *Journal of Clinical Microbiology*, 27, 731-734.

Fleet, G. (1992). Spoilage yeasts. *Critical Reviews in Biotechnology*, 12, 1-44.

Flores, M., Durá, M. A., Marco, A., & Toldra, F. (2004). Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science*, 68, 439-446.

Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry*, 45, 2178-2186.

Flores, M., Gianelli, M. P., Pérez-Juan, M., & Toldrá, F. (2007). Headspace concentration of selected dry-cured aroma compounds in model systems as affected by curing agents. *Food Chemistry*, 102, 488-493.

Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method from the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.

Foulquié Moreno, M. R., Sarantinopoulos, P., Tsakalidou, E., & De Vuyst, L. (2006). The role and application of enterococci in food and health. *International Journal of Food Microbiology*, 106, 1-24.

Fuentes, V., Estévez, M., Grèbol, N., Ventanas, J., & Ventanas, S. (2014). Application of time-intensity method to assess the sensory properties of Iberian dry-cured ham: effect of fat content and high-pressure treatment. *European Food Research and Technology*, 238, 397-408.

Fuentes, V., Utrera, M., Estévez, M., Ventanas, J., & Ventanas, S. (2014). Impact of high pressure treatment and intramuscular fat content on colour changes and protein and lipid oxidation in sliced and vacuum-packaged Iberian dry-cured ham. *Meat Science*, 97, 468-474.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, 85, 506-514.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry*, 41, 23-32.

García-Esteban, M., Ansorena, D., & Astiasarán, I. (2004). Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality. *Meat Science*, 67, 57-63.

García-Garrido, J. A., Quiles-Zafra, R., Tapiador, J., & de Castro, M. D. L., (2000). Activity of cathepsin B, D, H and L in Spanish dry-cured ham of normal and defective texture. *Meat Science*, 56, 1-6.

García-González, D. L., Tena, N., Aparicio-Ruiz, R., & Morales, M. T. (2008). Relationship between sensory attributes and volatile compounds qualifying dry-cured hams. *Meat Science*, 80, 315-325.

Garriga, M., Aymerich, M. T., Costa, S., Monfort, J. M., & Hugas, M. (2002). Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. *Food Microbiology*, 19, 509-518.

Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, 5, 451-457.

Giolitti, G., Cantoni, C., Bianchi, M., Renon, P., & Beretta, G. (1971). Microbiology and chemical changes in raw hams of Italian type. *Journal Applied Microbiology*, 34, 51-61.

Grasso, S., Brunton, N. P., Lyng, J. G., Lalor, F., & Monahan, F. J. (2014). Healthy processed meat products-Regulatory, reformulation and consumer challenges. *Trends in Food Science and Technology*, 39, 4-17.

Guillamón, J. M., Sabaté, J., Barrio, E., Cano, J., & Querol, A. (1998). Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Archives of Microbiology*, 169, 387-392.

Hamada, M., Shibata, C., Tamura, T., Nurkanto, A., Ratnakomala, S., Lisdiyanti, P., & Suzuki, K. (2016). *Cellulosimicrobium marinum* sp nov., an *actinobacterium* isolated from sea sediment. *Archives of Microbiology*, 198, 439-444.

Han, Y., Jiang, Y., Xu, X., Sun, X., Xu, B., & Zhou, G. (2011). Effect of high pressure treatment on microbial populations of sliced vacuum-packed cooked ham. *Meat Science*, 88, 682-688.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high pressure on enzymes related to food quality. *Trends in Food Science and Technology*, 9, 197-203.

Hereu, A., Bover-Cid, S., Garriga, M., & Aymerich, T. (2012). High hydrostatic pressure and biopreservation of dry-cured ham to meet the Food Safety Objectives for *Listeria monocytogenes*. *International Journal of Food Microbiology*, 154, 107-112.

Hinrichsen, L. L., & Andersen, H. J. (1994). Volatile compounds and chemical changes in cured pork: role of three halotolerant bacteria. *Journal of Agricultural and Food Chemistry*, 42, 1537-1542.

Hinrichsen, L. L., & Pedersen, S. B. (1995). Relationship among flavor, volatile compounds, chemical-changes, and microflora in Italian-type dry-cured ham during processing. *Journal of Agricultural and Food Chemistry*, 43, 2932-2940.

Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, 43, 99-107.

Huerta, T., Hernández, J., Guamis, B., & Hernández, E. (1988). Microbiological and physico-chemical aspects in dry-salted Spanish ham. *Zentralblatt für Mikrobiologie*, 143, 475-482.

Hugas, M., Garriga, M., & Monfort, J. M. (2002). New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, 62, 359-371.

Jofré, A., Aymerich, T., Grèbol, N., & Garriga, M. (2009). Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products. *LWT-Food Science and Technology*, 42, 924-928.

Jung, S., de Lamballerie-Anton, M., Taylor, R. G., & Ghoul, M. (2000). High-pressure effects on lysosome integrity and lysosomal enzyme activity in bovine muscle. *Journal of Agricultural and Food Chemistry*, 48, 2467-2471.

Jung, S., Ghoul, M., & de Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *LWT-Food Science and Technology*, 36, 625-631.

Justé, A., Van Trappen, S., Verreth, C., Cleenwerck, I., De Vos, P., Michiels, C. W., Lievens, B., & Willems, K. A. (2012). Characterization of *Tetragenococcus* strains from sugar thick juice reveals a new species, *Tetragenococcus osmophilus* sp. nov., and divides *Tetragenococcus halophilus* into two subspecies, *T. halophilus* subsp. *halophilus* subsp. nov. and *T. halophilus* subsp. *flandriensis* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 62, 129-137.

Kalchayanand, N., Sikes, A., Dunne, C. P., & Ray, B. (1998). Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. *Food Microbiology*, 15, 207-214.

Kato, M., & Hayashi, R. (1999). Effects of high pressure on lipids and biomembranes for understanding high-pressure-induced biological phenomena. *Bioscience, Biotechnology and Biochemistry*, 63, 1321-1328.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H., Yi, H., Won, S., & Chun, J. (2012). Introducing EzTaxon: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal Systematic and Evolutionary Microbiology*, 62, 716-721.

Kinsella, J. E., & Hwang, D. (1976). Biosynthesis of flavors by *Penicillium roqueforti*. *Biotechnology and Bioengineering*, 18, 927-938.

Koohmaraie, M., Seidemann, S. C., Schollmeyer, J. E., Dutson, T. R., & Crouse, J. D. (1987). Effect of post-mortem storage on Ca^{++} - dependent proteases, their inhibitor and myofibril fragmentation. *Meat Science*, 19, 187-196.

Kovács, G., Burghardt, J., Pradella, S., Schumann, P., Stackebrandt, E., & Màrialigeti, K. (1999). *Kocuria palustris* sp. nov, and *Kocuria rhizophila* sp. nov., isolated from the rhizoplane of the narrow-leaved cattail (*Typha angustifolia*). *International Journal of Systematic Bacteriology*, 49, 167-173.

Kruk, Z. A., Yun, H., Rutley, D. L., Lee, E. J., Kim, Y. J., & Jo, C. (2011). The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control*, 22, 6-12.

Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., Roepstorff, P., & Bendixen, E. (2003). Postmortem proteome changes of porcine muscle related to tenderness. *Journal of Agricultural and Food Chemistry*, 51, 6992-6997.

Landeta, G., Curiel, J., Carrascosa, A., Muñoz, R., & de las Rivas, B. (2013). Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products. *Meat Science*, 93, 387-396.

Landeta, G., Reverón, I., Carrascosa, A. V., de las Rivas, B., & Muñoz, R. (2011). Use of *recA* gene sequence analysis for the identification of *Staphylococcus equorum* strains predominant on dry-cured hams. *Food Microbiology*, 28, 1205-1210.

Langlois, B. E., & Kemp, J. D. (1974). Microflora of fresh and dry-cured hams as affected by fresh ham storage. *Journal of Animal Science*, 38, 525-531.

Lee, M., Kim, M. K., Vancanneyt, M., Swings, J., Kim, S. H., Kang, M. S. & Lee, S. T. (2005). *Tetragenococcus koreensis* sp. nov., a novel rhamnolipid-producing bacterium. *International Journal of Systematic and Evolutionary Bacteriology*, 55, 1409-1413.

Lindsay, D. S., Collins, M. V., Holliman, D., Flick, G. J., & Dubey, J. P. (2006). Effects of high-pressure processing on *Toxoplasma gondii* tissue cysts in ground pork. *Journal of Parasitology*, 92, 195-196.

López, M. O., de la Hoz, L., Cambero, M. I., Gallardo, E., Reglero, G., & Ordóñez, J. A. (1992).Volatile compounds of dry hams from Iberian pigs. *Meat Science*, 31, 267-277.

Lorenzo, J. M., Fontán, M. C. G., Gómez, M., Fonseca, S., Franco, I., & Carballo, J. (2012). Study of the *Micrococcaceae* and *Staphylococcaceae* throughout the manufacture of dry-cured Lacón (a Spanish traditional meat product) made without or with additives. *Journal Food Research*, 1, 200-211.

Lorido, L., Estévez, M., Ventanas, J., & Ventanas, S. (2015a). Salt and intramuscular fat modulate dynamic perception of flavour and texture in dry-cured hams. *Meat Science*, 107, 39-48.

Lorido, L., Estévez, M., Ventanas, J., & Ventanas, S. (2015b). Comparative study between Serrano and Iberian dry-cured hams in relation to the application of high hydrostatic pressure and temporal sensory perceptions. *LWT-Food Science and Technology*, 64, 1234-1242.

Luna, G., Aparicio, R., & García-González, D. R. (2006). A tentative characterization of white dry-cured hams from Teruel (Spain) by SPME–GC. *Food Chemistry*, 97, 621-630.

Lücke, F. K. (1986). Microbiological processes in the manufacture of dry sausage and raw ham. *Fleischwirtschaft*, 66, 1505-1509.

Lynch, J., Liu, Y. H., Mela, D. J., & MacFie, H. J. H. (1993). A time intensity study of the effect of oil mouthcoatings on taste perception. *Chemical Senses*, 182, 121-129.

Ma, H. J., & Ledward, D. A. (2004). High pressure / thermal treatment effects on the texture of beef muscle. *Meat Science*, 68, 347-355.

Ma, H. J., Ledward, D. A., Zamri, A. I., Frazier, R. A., & Zhou, G. H. (2007). Effects of high pressure / thermal treatment on lipid oxidation in beef and chicken muscle. *Food Chemistry*, 104, 1575-1579.

Mackey, B. M., Forestiere, K., Isaacs, N. S., Stenning, R., & Brooker, B. (1994). The effect of high hydrostatic pressure on *Salmonella thompson* and *Listeria monocytogenes* examined by electron microscopy. *Letters in Applied Microbiology*, 19, 429-432.

Mañas, P., & Mackey, B. M. (2004). Morphological and physiological changes induced by high hydrostatic pressure in exponential- and stationary-phase cells of *Escherichia coli*: relationship with cell death. *Applied and Environmental Microbiology*, 70, 1545-1554.

Marcos, B., Aymerich, T., Guardia, M. D., & Garriga, M. (2007). Assessment of high hydrostatic pressure and starter culture on the quality properties of low-acid fermented sausages. *Meat Science*, 76, 46-53.

Marín, M. E., de la Rosa, M. C., Carrascosa, A. V., & Cornejo, I. (1992). Microbiological and physicochemical aspects of spoiled dry-cured Spanish hams. *Fleischwirtschaft*, 72, 1600-1605.

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products. *Food Research International*, 94, 90-100.

Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology*, 110, 8-18.

Martín, A., Córdoba, J. J., Rodríguez, M. M., Nuñez, F., & Asensio, M. A. (2001). Evaluation of microbial proteolysis in meat products by capillary electrophoresis. *Journal of Applied Microbiology*, 90, 163-171.

Martín, L., Córdoba, J. J., Antequera, T., Timón, M. L., & Ventanas, J. (1998). Effects of salt and temperature on proteolysis during ripening of Iberian ham. *Meat Science*, 49, 145-153.

Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., & Picon, A. (2016a). Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry cured ham. *Meat Science*, 111, 130-138.

Martínez-Onandi, N., Rivas-Cañedo, A., Picon, A., & Nuñez, M. (2016b). Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage. *Meat Science*, 122, 101-108.

Martínez-Onandi, N., Castioni, A., San Martín, E., Rivas-Cañedo, A., Nuñez, M., Torriani, S., & Picon, A. (2017a). Microbiota of high-pressure-processed Serrano ham investigated by culture-dependent and culture-independent methods. *International Journal of Food Microbiology*, 241, 298-307.

Martínez-Onandi, N., Rivas-Cañedo, A., Ávila, M., Garde, S., Nuñez, M., & Picon, A. (2017b). Influence of physicochemical characteristics and high pressure processing on the volatile fraction of Iberian dry-cured ham. *Meat Science*, 131, 40-47.

Martínez-Onandi, N., Rivas-Cañedo, A., Picon, A., & Nuñez, M. (2017c). Influence of compositional characteristics, high pressure processing and prolonged refrigerated storage on the volatile compounds of Iberian dry-cured ham. *Meat Science*, (submitted).

Mauriello, G., Casaburi, A., Blaiotta, G., & Villani, F. (2004). Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. *Meat Science*, 67, 149-158.

May, L. A., Smiley, B., & Schmidt, M. G. (2001). Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. *Canadian Journal Microbiology*, 47, 829-841.

Min, D. B. S., Ina, K., Peterson, R. J., & Chang, S. S. (1977). The alkylbenzenes in roast beef. *Journal of Food Science*, 42, 503-505.

Moerman, F., Mertens, B., Demey, L., & Huyghebaert, A. (2001). Reduction of *Bacillus subtilis*, *Bacillus stearothermophilus* and *Streptococcus faecalis* in meat batters by temperature high hydrostatic pressure pasteurization. *Meat Science*, 59, 115-125.

Molina, I., & Toldrá, F. (1992). Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *Journal of Food Science*, 57, 1308-1310.

Monte, E., Villanueva, J. R., & Domínguez, A. (1986). Fungal profiles of Spanish country-cured hams. *Intentional Journal of Food Microbiology*, 3, 355-359.

Morales, P., Calzada, J., & Nuñez, M. (2006). Effect of high-pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. *Journal of Food Protection*, 69, 2539-2543.

Motilva, M. J., & Toldrá, F. (1993). Effect of curing agents and water activity on pork muscle and adipose subcutaneous tissue lipolytic activity. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 196, 228-232.

Motilva, M. J., Toldrá, F., & Flores, J. (1992). Assay of lipase and esterase activities in fresh pork meat and dry-cured ham. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 195, 446-450.

Motilva, M. J., Toldrá, F., Nieto, P., & Flores, J. (1993). Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chemistry*, 48, 121-125.

Muriel, E., Antequera, T., Petrón, M. J., Andrés, A. I., & Ruiz, J. (2004). Volatile compounds in Iberian dry-cured loin. *Meat Science*, 68, 391-400.

Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: A review. *Grasas y Aceites*, 63, 432-454.

Narváez-Rivas, M., Gallardo, E., & León-Camacho, M. (2015). Evolution of volatile hydrocarbons from subcutaneous fat during ripening of Iberian dry-cured ham. A tool to differentiate between ripening periods of the process. *Food Research International*, 67, 299-307.

Narváez-Rivas, M., Gallardo, E., Ríos, J. J., & León-Camacho, M. (2010). A tentative characterization of volatile compounds from Iberian dry-cured ham according to different anatomical locations. A detailed study. *Grasas y Aceites*, 61, 369-377.

Narváez-Rivas, M., Vicario, I. M., Constante, E. G., & León-Camacho, M. (2007). Changes in the concentrations of free fatty acid, monoacylglycerol, and diacylglycerol in the subcutaneous fat of Iberian ham during the dry-curing process. *Journal of Agricultural and Food Chemistry*, 55, 10953-10961.

Ng, W. F., Langlois, B. E., & Moody, W. G. (1997). Fate of selected pathogens in vacuum-packaged dry-cured (country-style) ham slices stored at 2 and 25 °C. *Journal of Food Protection*, 60, 1541-1547.

Norton, T., & Sun, D. W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, 1, 2-34.

Núñez, F., Rodríguez, M. M., Bermúdez, E., Córdoba, J. J., & Asensio, M. A. (1996a). Composition and toxigenic potential of mould population on dry-cured Iberian ham. *International Journal of Food Microbiology*, 32, 185-197.

Núñez, F., Rodríguez, M. M., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1996b). Yeast population during ripening of dry-cured Iberian ham. *International Journal of Food Microbiology*, 29, 271-280.

Ohmori, T., Shigehisa, T., Taji, S., & Hayashi, R. (1992). Biochemical effects of high hydrostatic pressure on the lysosome and proteases involved in it. *Bioscience, Biotechnology and Biochemistry*, 56, 1285-1288.

Ordoñez, J. A., Hierro, E. M., Bruna, J. M., & De la Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutritio*n, 39, 329-367.

Orlien, V., Hansen, E., & Skibsted, L. H. (2000). Lipid oxidation in high-pressure processed chicken breast muscle during chill storage: critical working pressure in relation to oxidation mechanism. *European Food Research and Technology*, 211, 99-104.

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science*, 74, 44-58.

Ouali, A., & Talmant, A. (1990). Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Science*, 28, 331-348.

Patterson, M. F. (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, 98, 1400-1409.

Patterson, M. F., Quinn, M., Simpson, R., & Gilmour, A. (1995). Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection*, 58, 524-529.

Ramírez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry*, 55, 1923-1931.

Rastogi, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, 47, 69-112.

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT-Food Science and Technology*, 44, 1251-1260.

Rico, E., Toldrá, F., & Flores, J. (1991a). Assay of cathepsin D activity in fresh pork muscle and dry-cured ham. *Meat Science*, 29, 287-293.

Rico, E., Toldrá, F., & Flores, J. (1991b). Effect of dry-curing process parameters on pork muscle cathepsins B, H and L activities. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 193, 541-544.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009a). Volatile compounds in dry-cured Serrano ham subjected to high pressure processing. Effect of the packaging material. *Meat Science*, 82, 162-169.

Rivas-Cañedo, A., Fernández-García, E., & Nuñez, M. (2009b). Volatile compounds in fresh meats subjected to high pressure processing: Effect of the packaging material. *Meat Science*, 81, 321-328.

Rivas-Cañedo, A., Juez-Ojeda, C., Nuñez, M., & Fernández-García, E. (2011). Effects of high pressure processing on the volatile compounds of sliced cooked pork shoulder during refrigerated storage. *Food Chemistry*, 124, 749-758.

Rodríguez, M., Nuñez, F., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1996). Gram-positive, catalase-positive cocci of dry-cured Iberian ham and their enterotoxigenic potential. *Applied and Environmental Microbiology*, 62, 1897-1902.

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, M. E., & Asensio, M. A. (1998). Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. *Journal of Applied Microbiology*, 85, 905-912.

Rodríguez, M., Núñez, F., Córdoba, J. J., Sanabria, C., Bermúdez, E., & Asensio, M. A. (1994). Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. *International Journal of Food Microbiology*, 85, 905-912.

Rohlf, F. J., & Sokal, R. R. (1969). Statistical tables, pp. 157-158. Editorial W. H. Freeman & Co., Ltd., San Francisco (EE. UU.)

Rojas, F. J., Jodral, M., Gosalvez, F., & Pozo, R. (1991). Mycoflora and toxigenic *Aspergillus flavus* in Spanish dry-cured ham. *International Journal of Food Microbiology*, 13, 249-255.

Röling, W. F. M., & van Verseveld, H. W. (1997). Growth, maintenance and fermentation pattern of the salt-tolerant lactic acid bacterium *Tetragenococcus halophila* in anaerobic glucose limited retention cultures. *Antonie van Leeuwenhoek*, 72, 239-243.

Rosell, C. M., & Toldrá, F. (1998). Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and White pigs. *Journal of the Science of Food and Agriculture*, 76, 117-122.

Rubio, B., Martínez, B., García-Cachan, M. D., Rovira, J., & Jaime, I. (2007a). Effect of high pressure preservation on the quality of dry cured beef "Cecina de Leon". *Innovative Food Science and Emerging Technologies*, 8, 102-110.

Rubio, B., Martínez, B., García-Cachán, M. D., Rovira, J., & Jaime, I. (2007b). The effects of high pressure treatment and of storage periods on the quality of vacuum-packed "salchichón" made of raw material enriched in monounsaturated and polyunsaturated fatty acids. *Innovative Food Science and Emerging Technologies*, 8, 180-187.

Ruiz, J., Cava, R., Ventanas, J., & Jensen, M. T. (1998). Headspace Solid Phase Microextraction for the analysis of volatiles in a meat product: Dry-cured Iberian ham. *Journal of Agricultural and Food Chemistry*, 46, 4688-4694.

Ruiz, J., García, C., Muriel, E., Andrés, A. I., & Ventanas, J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science*, 61, 347-354.

Ruiz, J., Ventanas, J., & Cava, R. (2001). New device for direct extraction of volatile in solid samples using SPME. *Journal of Agricultural and Food Chemistry*, 49, 5115-5121.

Ruiz, J., Ventanas, J., Cava, R., Andrés, A., & García, C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science*, 52, 19-27.

Ruiz, J., Ventanas, J., Cava, R., Timón, M. L., & García, C. (1998). Sensory characteristics of Iberian ham: Influence of processing time and slice location. *Food Research International*, 31, 53-58.

Sabio, E., Vidal-Aragón, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from South European countries. *Food Chemistry*, 61, 493-503.

Samelis, J., Kakouri, A., & Rementzis, J. (2000). Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4 °C. *Food Microbiology*, 17, 329-340.

San Martin, M. F., Barbosa-Cánovas, G. V., & Swanson, B. G. (2002). Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition*, 42, 627-645.

Sanabria, C., Carrascosa, A. V., Sabio, E., & Fallola, A. (1997). HACCP for Iberico dry-cured ham. *Fleischwirtschaft*, 77, 1006-1008.

Sánchez-Peña, C. M., Luna, G., García-González, D. L., & Aparicio, R. (2005). Characterization of French and Spanish dry-cured hams: Influence of the volatile from the muscles and the subcutaneous fat quantified by SPME-GC. *Meat Science*, 69, 635-645.

Santos-Garcés, E., Gou, P., García-Gil, N., Arnau, J., & Fulladosa, E. (2010). Non-destructive analysis of a_w, salt and water in dry-cured hams during drying process by means of computed tomography. *Journal of Food Engineering*, 101, 187-192.

Sárraga, C., Gil, M., Arnau, J., Monfort, J. M., & Cussó, R. (1989). Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Science*, 25, 241-249.

Serra, X., Sárraga, C., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., & Arnau, J. (2007a). High pressure applied to frozen ham at different process stages. 1. Effect on the final physicochemical parameters and on the antioxidant and proteolytic enzyme activities of dry-cured ham. *Meat Science*, 75, 12-20.

Serra, X., Grèbol, N., Guàrdia, M. D., Guerrero, L., Gou, P., Masoliver, P., Gassiot, M., Sárraga, C., Monfort, J.M., & Arnau, J. (2007b). High pressure applied to frozen ham at different process stages. 2. Effect on the sensory attributes and on the colour characteristics of dry-cured ham. *Meat Science*, 75, 21-28.

Shahidi, F., Rubin, L. J., & D'Souza, L. A. (1986). Meat flavor volatiles-A review of the composition, techniques of analysis, and sensory evaluation. *Critical Reviews in Food Science and Nutrition*, 24, 141-243.

Shigehisa, T., Ohmori, T., Saito, A., Taji, S., & Hayashi, R. (1991). Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. *International Journal of Food Microbiology*, 12, 207-215.

Simoncini, N., Rotelli, D., Virgili, R., & Quintavalla, S. (2007). Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. *Food Microbiology*, 24, 577-584.

Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, 9, 152-158.

Solomon, E. B., & Hoover, D. G. (2004). Inactivation of *Campylobacter jejuni* by high hydrostatic pressure. *Letters in Applied Microbiology*, 38, 505-509.

Spinnler, H. E., Berger, C., Lapadatescu, C., & Bonnarme, P. (2001). Production of sulfur compounds by several yeasts of technological interest for cheese ripening. *International Dairy Journal*, 11, 245-252.

Stahnke, L. H. (1994). Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Science*, 38, 39-53.

Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels - Part II. Volatile components. *Meat Science*, 41, 193-209.

Sutic, M., Ayres, J. C., & Koehler, P. E. (1972). Identification and aflatoxin production of moulds isolated from country cured hams. *Journal of Applied Microbiology*, 23, 656-658.

Takeuchi, M., Fang, C.-X., & Yokota, A. (1995). Taxonomic Study of the Genus *Brachybacterium*: Proposal of *Brachybacterium conglomeratum* sp. nov., nom. rev., *Brachybacterium paraconglorneratum* sp. nov., and *Brachybacterium rhamnosum* sp. nov. *International Journal of Systematic Bacteriology*, 45, 160-168.

Tewari, G., Jayas, D. S., & Holley, R. A. (1999). High pressure processing of foods: an overview. *Sciences des Aliments*, 19, 619-661.

Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A., & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science*, 58, 85-91.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science*, 49, S101-S110.

Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. *Trends in Food Science and Technology*, 17, 164-168.

Toldrá, F., Aristoy, M. C., & Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International*, 33, 181-185.

Toldrá, F., & Etherington, D. J. (1988). Examination of cathepsins B, D, H and L activities in dry-cured hams. *Meat Science*, 23, 1-7.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, 38, 331-352.

Toldrá, F., Flores, M., & Sanz, Y. (1997). Dry-cured ham flavour: Enzymatic generation and process influence. *Food Chemistry*, 59, 523-530.

Toldrá, F., Rico, E., & Flores, J. (1992). Activity of pork muscle proteases in model cured meat systems. *Biochimie*, 74, 291-296.

Toldrá, F., Rico, E., & Flores, J. (1993). Cathepsin B, D, H and L activities in the processing of dry-cured ham. *Journal of the Science of Food and Agriculture*, 62, 157-161.

Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Journal of Food Science*, 57, 813-815.

Ventanas, J., Ruiz, J., & Córdoba, J. J. (2001). El jamón curado de cerdo Ibérico: descripción del proceso tradicional de elaboración. En: Tecnología del jamón Ibérico. De los sistemas tradicionales a la explotación racional del sabor y el aroma, pp. 45-71. Editor: J. Ventanas Barroso. Editorial Mundi-Prensa, Madrid.

Ventanas, S., Estévez, M., Andrés, A. I., & Ruiz, J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscular fat contents using SPME-DED. *Meat Science*, 79, 172-180.

Vermassen, A., Dordet-Frisoni, E., de la Foye, A., Micheau, P., Laroute, V., Leroy. S., & Talon, R. (2016). Adaptation of *Staphylococcus xylosus* to nutrients and osmotic stress in a salted meat model. *Frontiers in Microbiolology*, 7, 87.

Walter, J., Tannock, G. W., & Tilsala-Timisjarvi, A. (2000). Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Applied and Environmental Microbiology*, 66, 297-303.

Wang, J. M., Jin, G. F., Zhang, W. G., Ahn, D. U., & Zhang, J. H. (2012). Effect of curing salt content on lipid oxidation and volatile flavour compounds of dry-cured turkey ham. *LWT-Food Science and Technology*, 48, 102-106.

White, T. J., Bruns, T. D., Lee, S., & Taylor, J. (1990). Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. En: PCR Protocols: A Guide to Methods and Applications, pp. 315-322. Editores: M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White. Editorial Academic Press, Nueva York (EE. UU.).

Yoon, J. H., Kang, S. J., Schumann, P., & Oh, T. K. (2007). *Cellulosimicrobium terreum* sp nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 57, 2493-2497.

Yuste, J., Capellas, M., Pla, R., Fung, D. Y. C., & Mor-Mor, M. (2001). High pressure processing for food safety and preservation: a review. *Journal of Rapid Methods & Automation in Microbiology*, 9, 1-10.

Yuste, J., Pla, R., Capellas, M., Ponce, E., & Mor-Mur, M. (2000). High-pressure processing applied to cooked sausages: bacterial populations during chilled storage. *Journal of Food Protection*, 63, 1093-1099.

Zhang, J. H., Zhen, Z. Y., Zhang, W. G., Zeng, T., & Zhou, G. H. (2009). Effect of intensifying high-temperature ripening on proteolysis, lipolysis and flavor of Jinhua ham. *Journal of the Science of Food and Agriculture*, 89, 834-842.

Zhang, J. H., Jin, G. F., Wang, J. M., & Zhang, W. G. (2011). Effect of intensifying high-temperature ripening on lipolysis and lipid oxidation of Jinhua ham. *LWT-Food Science and Technology*, 44, 473-479.

Zhou, G. H., & Zhao, G. M. (2007). Biochemical changes during processing of traditional Jinhua ham. *Meat Science*, 77, 114-120.

11. Resumen ampliado

11.1. Introducción

El proceso de elaboración del jamón curado consta de las etapas de salado, post-salado y secado o maduración, precedidas de un acondicionamiento previo consistente en la recepción, clasificación y pre-salado de los perniles (Toldrá et al., 1997). Este proceso de elaboración es válido en líneas generales tanto para el jamón Serrano como para el jamón Ibérico, aunque existen claras diferencias entre ellos debidas a la raza de cerdo de que provienen, la alimentación y el manejo de los animales, algunas particularidades del proceso de elaboración y la duración del periodo de maduración.

Uno los atributos de calidad más importantes en el jamón curado es su aroma intenso y persistente debido principalmente a un gran número de compuestos volátiles con bajo umbral de detección olfativa (Flores et al., 1997). Los compuestos volátiles y no volátiles responsables del olor, aroma y sabor del jamón curado se generan fundamentalmente durante la etapa de maduración, que dura por lo general de 9 a 12 meses en el jamón Serrano y de 18 a 24 meses en el jamón Ibérico.

Las principales reacciones bioquímicas que tienen lugar en el jamón a lo largo de su elaboración y maduración son mayoritariamente de tipo enzimático, destacando la hidrólisis de proteínas musculares (proteolisis), la hidrólisis de triglicéridos y fosfolípidos (lipolisis) y, en menor medida, la hidrólisis de glúcidos (glucolisis) y la degradación de nucleótidos. Con los productos de hidrólisis como sustratos tienen lugar reacciones químicas secundarias tales como las reacciones de Maillard, la degradación de Strecker y la oxidación de lípidos y proteínas. Todas estas reacciones ocurren de manera simultánea y su intensidad depende de las condiciones existentes durante la elaboración y maduración del jamón (Flores et al., 1997).

Con el objetivo de satisfacer la creciente demanda por parte de los consumidores de alimentos más frescos, con menos aditivos y más seguros desde el punto de vista microbiológico, la industria alimentaria desarrolla nuevas tecnologías de procesado y conservación. El tratamiento por altas presiones hidrostáticas (APH), clasificado como una pasteurización no térmica y de procesado mínimo, es una de las tecnologías emergentes para el procesado de alimentos que más ha crecido en los últimos

años. A diferencias de otras técnicas de conservación de alimentos, las APH generan una percepción positiva por parte del consumidor (Cardello et al., 2007) y constituyen una herramienta muy útil para las empresas exportadoras de productos cárnicos envasados. Su principal objetivo es la inactivación de microorganismos, tanto patógenos como alterantes, y enzimas para aumentar la seguridad y la estabilidad del alimento.

El fundamento de las APH consiste en someter a los alimentos envasados a una presión hidrostática empleando un fluido transmisor (Norton & Sun, 2008**)**. La tecnología de APH se basa en dos principios físicos, la ley de la isostática y el principio de Le Châtelier. Los equipos industriales de APH suelen constar de una cámara de presurización, un sistema generador de presión y un dispositivo de control de la temperatura (San Martín et al., 2002). El alimento, una vez envasado herméticamente en un material flexible, se introduce en la cámara de presurización. Esta se cierra, se llena con el fluido transmisor y se aumenta la presión hasta alcanzar el nivel deseado. Después, se detiene el bombeo de fluido y se cierran las válvulas, manteniéndose la presión constante durante el tiempo de tratamiento deseado. Actualmente las condiciones más frecuentes en la industria cárnica son de 600 MPa durante 5-10 min a 10-25 ºC.

El efecto letal del tratamiento de APH sobre los microorganismos es uno de los principales atractivos que tiene esta tecnología para su uso por la industria cárnica. Las APH consiguen la inactivación de microorganismos y enzimas sin apenas alterar el contenido nutricional y las características sensoriales de los alimentos (Hoover et al., 1989; Norton & Sun, 2008). Los cambios producidos en las células microbianas tras la aplicación de este tratamiento incluyen alteraciones en la membrana y la pared celular, en su morfología, en las actividades enzimáticas y en los mecanismos genéticos (Hoover et al., 1989; Smelt, 1998; Tewari et al., 1999). El grado de inactivación microbiana depende de parámetros tales como el tipo de microorganismo y su morfología, su estado fisiológico, la presión, el tiempo y la temperatura aplicada durante el proceso, así como el pH y la composición del alimento.

El tratamiento de la carne y los productos cárnicos por APH puede afectar a algunas de sus características relacionadas con la calidad organoléptica, tales como la textura, el color y el nivel de oxidación lipídica. En el caso concreto del jamón curado, se han llevado a cabo diversos estudios sobre el efecto de las APH sobre la microbiota, el color, la textura, las actividades enzimáticas, la oxidación lipídica, los compuestos químicos y las características sensoriales del jamón curado.

Respecto al efecto de las APH sobre la microbiota del jamón curado, Garriga et al. (2004) observaron que en jamón curado loncheado, envasado al vacío y tratado a 600 MPa durante 6 min a 31 ºC, la microbiota total disminuía al menos 2 unidades logarítmicas y se mantenían en niveles bajos durante el almacenamiento en refrigeración. Morales et al. (2006) investigaron la inactivación de *L. monocytogenes* en jamón loncheado envasado al vacío y tratado a 450 MPa durante 10 min a 12 ºC, registrando descensos de 1,50 y 1,16 unidades logarítmicas en jamón Serrano y jamón Ibérico, respectivamente. Clariana et al. (2011) comprobaron que en el jamón curado loncheado, envasado al vacío y tratado a 600 MPa durante 6 min a 15 ºC, se producía una disminución significativa en los niveles de microorganismos aerobios totales a lo largo de los 50 días de almacenamiento en refrigeración. De Alba et al. (2012) observaron disminuciones de 1,06, 2,54 y 4,32 unidades logarítmicas en los niveles de *Salmonella* Enteritidis en jamón Serrano tratado a 400, 500 y 600 MPa, respectivamente, durante 5 min a 12 ºC mientras que de Alba et al. (2013) registraron descensos de 0,25 y 1,28 unidades logarítmicas en los niveles de *E. coli* O157:H7 en jamón Serrano inmediatamente después del tratamiento a 400 y 500 MPa durante 10 min a 12 ºC. Bover-Cid et al. (2015) estudiaron la influencia de la actividad de agua (a_w) y el contenido de grasa sobre la reducción de *L. monocytogenes* en jamón curado tras la aplicación de APH (347 - 852 MPa, 5 min, 15 ºC), observando que valores bajos de a_w tenían un efecto protector a cualquier nivel de presión y que el alto contenido en grasa tenía un efecto protector por encima de 700 MPa mientras que a menor presión los contenidos de grasa superiores al 30 % favorecían la inactivación de *L. monocytogenes*. Bover-Cid et al. (2017) comprobaron que con una a_w de 0,88 la reducción de los niveles de *Salmonella enterica* apenas variaba

al aumentar la presión mientras que con una a_w de 0,96 la reducción de *Salmonella entérica* aumentaba considerablemente al aumentar la presión, sin que se observase un efecto protector de la grasa sobre el patógeno.

Por lo que respecta a la textura en el jamón curado, Serra et al. (2007b) comprobaron que tratamientos a 400 y 600 MPa causaba pequeñas variaciones en la textura mientras que Clariana et al. (2011) observaron que tras un tratamiento a 600 MPa durante 6 min a 15-32 ºC aumentaban la dureza y la fuerza de masticación. De Alba et al. (2012) registraron valores más bajos de los parámetros de textura en las muestras tratadas por APH que en las muestras control, siendo mayor el efecto del tiempo de almacenamiento que el efecto del tratamiento por APH.

El color del jamón curado es una de las características organolépticas más apreciadas por los consumidores. Algunos autores (Andrés et al., 2004, 2006) han observado que tras el tratamiento del jamón curado por APH se producían incrementos del parámetro L* y descensos del parámetro a*. En jamón Serrano e Ibérico tratados a 450 MPa durante 10 min a 12 ºC no se registraron variaciones en los parámetros L* y a* mientras que el parámetro b* aumentaba, aunque solamente en el jamón Ibérico (Morales et al., 2006). Un tratamiento de APH a 600 MPa ocasionaba un aumento del parámetro L* del jamón curado y una reducción de la intensidad del color (Clariana et al., 2011). Los diferentes efectos encontrados en el color del jamón curado tras el tratamiento de APH se han atribuido a diferencias iniciales en la composición del pigmento del jamón, aunque las variaciones de los parámetros de color respecto del control después de tratamientos a 400 y 600 MPa eran pequeñas (Serra et al., 2007b).

La oxidación lipídica durante la maduración del jamón curado contribuye al desarrollo de las características de olor, aroma y sabor del producto (Toldrá & Flores, 1998), por lo que el efecto de las APH sobre la oxidación lipídica es un aspecto importante que se debe valorar. La actividad de las enzimas antioxidantes (catalasa, glutatión peroxidasa y superóxido dismutasa) disminuyó ligeramente en jamón en las etapas iniciales del proceso de elaboración tras el tratamiento de APH (Serra et al.,

2007a). Sin embargo, no se observó un efecto de las APH sobre las actividades enzimáticas antioxidante en el jamón curado (Clariana et al., 2011). En jamón Ibérico loncheado, las APH tuvieron un efecto reducido sobre la oxidación lipídica a presiones inferiores a 300 MPa pero el efecto aumentaba a presiones superiores (Andrés et al., 2004, 2006). Al tratar jamón Ibérico loncheado a 200 y 300 MPa durante 15 o 30 min se observó un aumento de la oxidación lipídica al aumentar la presión y el tiempo de tratamiento, pero a los 90 días de almacenamiento en refrigeración las diferencias entre muestras control y tratadas no fueron significativas (Cava et al., 2009). Al aplicar un tratamiento de 600 MPa durante 6 min a 12 ºC en jamón Ibérico, seguido de almacenamiento en refrigeración durante 30 días con ciclos de luz / oscuridad de 12 h, se observó un aumento significativo en los niveles de oxidación de lípidos y proteínas (Fuentes et al., 2010). En jamón Serrano loncheado tratado a 600 MPa durante 6 min a 15 - 32 ºC se mantuvo la estabilidad oxidativa de los lípidos (Clariana et al., 2011). Sin embargo, en jamón Serrano tratado a 400, 500 y 600 MPa los niveles de oxidación de lípidos aumentaron tras 30 y 60 días de almacenamiento en refrigeración (de Alba et al., 2012).

Los compuestos volátiles del jamón curado también han sido objeto de diversos estudios. En jamón Serrano, Rivas-Cañedo et al. (2009) investigaron el efecto de un tratamiento a 400 MPa durante 10 min a 12 ºC, seguido de almacenamiento en refrigeración de 3 días, comprobando que la mayoría de los compuestos afectados por el tratamiento tenían niveles menores en las muestras tratadas, a excepción de la 2-heptanona que incrementó su nivel. No registraron un aumento en los niveles de los compuestos volátiles procedentes de reacciones de oxidación lipídica tras el tratamiento. Por el contrario, en jamón Ibérico, Fuentes et al. (2010) observaron tras el tratamiento a 600 MPa durante 6 min a 12 ºC, seguido de almacenamiento en refrigeración durante 30 días, un aumento significativo en los niveles de aldehídos lineales. El efecto del tratamiento de APH sobre los compuestos volátiles derivados de las reacciones de Maillard y la degradación de Strecker es variable. Rivas-Cañedo et al. (2009) no observaron efectos significativos sobre ninguno de los compuestos volátiles procedentes de estas rutas. Por el contrario, Fuentes et al. (2010) registraron una disminución de los niveles de 2-

metilbutanal y 3-metilbutanal en el formato de envase convencional, un aumento de pentanal en el formato loncheado apilado en vertical, un aumento de heptanal en el loncheado convencional, y un aumento de hexanal en los dos formatos de loncheado entre el jamón tratado y el no tratado por APH.

El tratamiento de APH no parece tener una gran influencia sobre las características de olor y sabor en el jamón curado. Morales et al. (2006) no encontraron diferencias significativas en el sabor del jamón Serrano e Ibérico después de un tratamiento de 450 MPa durante 10 min a 12 ºC. Igualmente, Clariana et al. (2011) encontraron que en jamón Serrano loncheado tratado a 600 MPa la intensidad del olor disminuía inmediatamente después del tratamiento pero que al cabo de 50 días el jamón tratado mostraba una mayor intensidad de olor y una mayor retención de compuestos del aroma. Estos autores observaron que la percepción del sabor salado era mayor en el jamón tratado, atribuyéndolo a una mayor accesibilidad de los iones de sodio en dichas muestras. Por otra parte, Fuentes et al. (2014) comprobaron que las muestras de jamón ibérico tratadas a 600 MPa tenían una mayor intensidad de sabor y un sabor más salado y más rancio que las no tratadas, según los resultados de un análisis sensorial dinámico llevado a cabo aplicando el método tiempo-intensidad.

11.2. Objetivos

Los objetivos de la presente Tesis doctoral fueron los siguientes:

- Investigar la influencia de la composición química y las altas presiones hidrostáticas (APH) sobre los compuestos volátiles presentes en jamón Serrano, a los 3 días y después de 5 meses de almacenamiento en refrigeración a 4 ºC.
- Estudiar el efecto de las APH sobre la microbiota del jamón Serrano con diferentes contenidos de grasa intramuscular y sal.

- Investigar la influencia de la composición química y las APH sobre los compuestos volátiles presentes en jamón Ibérico, a los 3 días y después de 5 meses de almacenamiento en refrigeración a 4 ºC.
- Estudiar el efecto de las APH sobre la microbiota del jamón Ibérico con diferentes contenidos de grasa intramuscular y sal.

11.3. Materiales y Métodos

El estudio se llevó a cabo sobre 60 jamones Serranos y 60 jamones Ibéricos. La elaboración de jamón Serrano se llevó a cabo en el Institut de Recerca i Tecnologia Agroalimentàries (IRTA, Monells, España) y la elaboración de jamón Ibérico en una industria cárnica de Extremadura. Los jamones enteros se seleccionaron por su contenido en grasa mediante tecnología de sensores de resonancia magnética utilizando un tomógrafo computerizado (Scanner model HiSpeed Zx/i, GE Healthcare, Barcelona, España). Para la obtención de las muestras se extrajo la "maza" del jamón, la cual se dividió en dos partes, una de las cuales se utilizó como control y la otra se sometió a un tratamiento de APH a 600 MPa durante 6 min a 21 ºC. La mitad de las muestras, tanto controles sin tratar como tratadas por APH, se analizaron a los 3 días y la otra mitad después de 5 meses en refrigeración a 4 ºC.

El contenido en cloruro se determinó mediante el método de Volhard (AOAC, 2000) y el contenido de grasa intramuscular mediante extracción con cloroformo-metanol (Folch et al., 1957). La a_w de las muestras se midió con un equipo AquaLab Series 3-equipment (Decagon Devices, Inc., Pullman, WA, EE.UU.).

Para el estudio de la fracción volátil, los compuestos volátiles del jamón Serrano e Ibérico se extrajeron mediante microextracción en fase sólida (SPME) y se analizaron mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS).

Para el estudio de la microbiota del jamón Serrano e Ibérico se realizaron análisis microbiológicos en medios sólidos para bacterias mesófilas totales (PCA, 30 ºC), bacterias psicrotrofas

(PCA, 8 ºC), enterobacteriáceas (VRBG), bacterias lácticas (MRS), enterococos (KAA), micrococáceas (MSA), estafilococos (BP), y mohos y levaduras (SDA). También se determinó la presencia-ausencia de *Listeria monocytogenes* y *Salmonella* spp. en 25 g de muestra.

La biodiversidad de la población microbiana del jamón Serrano se estudió mediante electroforesis en gel con gradiente de desnaturalización (DGGE). La biodiversidad de la microbiota del jamón Ibérico se estudió sobre aislados microbianos identificados por técnicas moleculares tras la amplificación por PCR de una región de unos 800 pares de bases del gen *16S rDNA*.

11.4. Resultados

11.4.1. Jamón Serrano

En la fracción volátil del jamón Serrano a los 3 días en refrigeración se identificaron 100 compuestos: 7 ácidos, 21 alcoholes, 7 aldehídos, 6 alcanos, 5 ésteres, 10 cetonas, 20 compuestos bencénicos, 7 compuestos azufrados, 6 furanos, 2 furanonas, 3 pirazinas, 2 terpenos y 4 compuestos misceláneos. En jamón Serrano después de 5 meses en refrigeración se identificaron 103 compuestos volátiles: 8 ácidos, 21 alcoholes, 7 aldehídos, 8 alcanos, 6 esteres, 9 cetonas, 18 compuestos bencénicos, 7 compuestos azufrados, 7 furanos, 2 furanonas, 4 pirazinas, 2 terpenos y 4 compuestos misceláneos.

La composición química del jamón Serrano a los 3 días en refrigeración afectó a algunos ácidos, alcoholes, aldehídos ramificados, cetonas, compuestos bencénicos y azufrados, furanos, furanonas y pirazinas. El contenido de grasa intramuscular y la concentración de sal fueron los parámetros composicionales que más influencia tuvieron sobre la fracción volátil del jamón Serrano a los 3 días en refrigeración. El contenido en grasa intramuscular influyó en los niveles de 51 compuestos volátiles. Los jamones Serranos con valores altos de grasa intramuscular mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica (ácidos y compuestos bencénicos), de las reacciones de Maillard (furanos y pirazinas), de la degradación de Strecker (aldehídos ramificados) y

del catabolismo de aminoácidos azufrados (compuestos azufrados) mientras que los jamones con valores bajos de grasa intramuscular mostraron niveles elevados de compuestos procedentes del metabolismo microbiano (2-alcoholes y alcoholes ramificados). La concentración de sal influyó significativamente en los niveles de 40 compuestos volátiles. Los jamones Serranos con alta concentración de sal mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica o de β-oxidación de ácidos grasos libres por mohos (alcoholes primarios y cetonas) y de compuestos procedentes del metabolismo microbiano (2-alcoholes y alcoholes ramificados) mientras que los jamones con baja concentración de sal mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (furanos y pirazinas). La relación sal en magro y la a_w influyeron significativamente en los niveles de 41 y 24 compuestos volátiles respectivamente. Los jamones Serrano con valores altos de la relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica o de β-oxidación de ácidos grasos libres por mohos (alcoholes primarios y cetonas) y de compuestos procedentes del metabolismo microbiano (alcoholes ramificados) mientras que los jamones con valores bajos de relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (pirazinas) y de la degradación de Strecker (aldehídos ramificados). Los jamones Serrano con valores altos de a_w mostraron niveles elevados de compuestos volátiles procedentes del metabolismo microbiano (2-alcoholes, alcoholes ramificados, ésteres y compuestos azufrados) mientras que los jamones con valores bajos de a_w mostraron niveles elevados de compuestos volátiles procedentes de la actividad enzimática.

La composición química del jamón Serrano después de 5 meses en refrigeración afectó principalmente a algunos ácidos, alcoholes, aldehídos, alcanos, cetonas, compuestos bencénicos y azufrados, ésteres y furanos. El contenido de grasa intramuscular fue el parámetro composicional que más influyó sobre la fracción volátil del jamón Serrano después de 5 meses en refrigeración influyendo significativamente en los niveles de 46 compuestos volátiles. Los jamones Serranos con valores altos de grasa intramuscular mostraron niveles elevados de compuestos procedentes de reacciones de

oxidación lipídica (ácidos y compuestos bencénicos), de reacciones de Maillard (furanos y pirazinas) y de la reacción de esterificación enzimática (ésteres) mientras que los jamones con valores bajos de grasa intramuscular mostraron niveles elevados de compuestos del metabolismo microbiano (2-alcoholes y alcoholes ramificados). La concentración de sal y la relación sal en magro influyeron significativamente en los niveles de 7 compuestos volátiles respectivamente. Los jamones Serrano con valores bajos de la concentración de sal y la relación sal en magro mostraron niveles elevados de compuestos procedentes de reacciones de Maillard (pirazinas). La a_w influyó significativamente en 12 compuestos volátiles. Los jamones Serrano con valores medios de a_w mostraron niveles elevados de compuestos procedentes del metabolismo microbiano (2-alcoholes).

El tratamiento de APH influyó significativamente en los niveles de 8 de los 100 compuestos volátiles identificados en el jamón Serrano a los 3 días en refrigeración. Dos compuestos azufrados alcanzaron mayores niveles en los jamones Serranos tratados por APH y, por el contrario, 4 ésteres y otros 2 compuestos azufrados alcanzaron menores niveles en los jamones Serranos tratados por APH. En el jamón Serrano después de 5 meses en refrigeración, el tratamiento de APH influyó significativamente en los niveles de 21 de los 103 compuestos volátiles identificados. En los jamones Serranos tratados por APH, 3 alcoholes, 2 alcanos, 4 cetonas, 1 compuesto bencénico y 2 compuestos azufrados alcanzaron niveles más elevados y, por el contrario, 5 alcoholes, 2 ésteres y 2 compuestos azufrados alcanzaron niveles inferiores.

De los 100 compuestos identificados en la fracción volátil del jamón Serrano a los 3 días en refrigeración y de los 103 compuestos identificados en la fracción volátil del jamón Serrano después de 5 meses en refrigeración, 95 fueron identificados tanto a los 3 días como a los 5 meses en refrigeración. Cinco compuestos (2-heptanol, 2-metil-2-buten-1-ol, 2,3-pentanodiona, estireno 1 y estireno 2) se identificaron solamente a los 3 días en refrigeración y 8 compuestos (ácido heptanoico,

1-propanol, 3-metil-2-butanol, decano, alcano ramificado, decanoato de etilo, 2-etilfurano y 2,3,5-trimetilpirazina) se identificaron solo después de 5 meses en refrigeración.

Los niveles totales de alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados, furanonas, terpenos y compuestos misceláneos disminuyeron ($P < 0,05$) durante la refrigeración en las muestras control de jamón Serrano. Los niveles totales de alcoholes, cetonas, terpenos y compuestos misceláneos disminuyeron ($P < 0,05$) y los niveles totales de furanos aumentaron ($P < 0,05$) durante la refrigeración en las muestras de jamón Serrano tratadas por APH. Respecto a los compuestos individuales, 45 compuestos en la fracción volátil del jamón Serrano resultaron afectados por el tiempo de almacenamiento en refrigeración en las muestras control, 46 compuestos resultaron afectados por el tiempo de almacenamiento en las muestras tratadas por APH y 42 compuestos resultaron afectados tanto en las muestras control como en las tratadas. Los niveles de 1-butanol, 1-pentanol, 1-octanol, 2-propanol, 2-heptanol, 2-metil-2-buten-1-ol, 2-metil-3-buten-2-ol, 2-butoxietanol, 1-metoxi-2-propanol, heptanal, 2-metilbutanal, dodecano, 2-propanona, 2-butanona, 2-pentanona, 2-heptanona, 2,3-pentadiona, 3-hidroxi-2-butanona, 4-metil-2-pentanona, etilbenceno, etil estireno 1, etil estireno 2, benzonitrilo, 4-metil-fenol, 3-fenil-2-propenal, 4-fenil-3-buten-2-ona, naftaleno, dioxido de sulfuro, 2,3,5-trimetilfurano, 2,3-dihidro-4-metilfurano, α-pineno, *p*-nitrofenil hexanoato disminuyeron durante la refrigeración mientras que los niveles de ácido hexanoico, 1-propanol, 3-metil-2-butanol, nonanal, dodecanal, decano, alcano ramificado, decanoato de etilo, 5-metil-3-heptanona, 2-etilfurano y 2,3,5-trimetilpirazina aumentaron durante la refrigeración. Tres compuestos volátiles resultaron afectados solamente en las muestras control: 3-metilbutanoato de etilo y 2-octanona disminuyeron durante la refrigeración mientras que el ácido pentanoico aumentó durante la refrigeración. Cuatro compuestos volátiles resultaron afectados solamente en las muestras tratadas por APH: 1-hexanol y α-pineno disminuyeron durante la refrigeración mientras que hexano y undecano aumentaron durante la refrigeración.

La composición química del jamón Serrano a los 3 días en refrigeración influyó significativamente sobre los niveles de aerobios mesófilos, psicrotrofos, bacterias lácticas, y mohos y levaduras. La grasa intramuscular influyó significativamente en los niveles de aerobios mesófilos, psicrotrofos, y mohos y levaduras en los jamones Serranos control. Los jamones con bajo contenido en grasa intramuscular mostraron mayores niveles de microorganismos. La concentración de sal fue el parámetro composicional que más influyó sobre los niveles de microorganismos en el jamón Serrano a los 3 días en refrigeración influyendo significativamente en los niveles de aerobios mesófilos, psicrotrofos, bacterias lácticas, y mohos y levaduras en los jamones Serrano control. Los jamones con concentraciones altas de sal mostraron mayores niveles de aerobios mesófilos, psicrotrofos, y mohos y levaduras y los de concentraciones intermedias de sal mostraron mayores niveles de bacterias lácticas. La relación sal en magro influyó significativamente en los niveles de aerobios mesófilos, psicrotrofos, y mohos y levaduras en los jamones Serranos control. Los jamones con elevada relación sal en magro mostraron mayores niveles de aerobios mesófilos, psicrotrofos, y mohos y levaduras. La a_w no afectó significativamente a los niveles de ningún grupo microbiano ni en los jamones Serranos control ni en los tratados por APH.

La composición química del jamón Serrano después de 5 meses en refrigeración influyó significativamente sobre los niveles de aerobios mesofilos, psicrotrofos, micrococáceas, y mohos y levaduras. El contenido en grasa intramuscular influyó significativamente en los niveles de aerobios mesófilos, psicrotrofos, micrococáceas, y mohos y levaduras en las muestras control. Los jamones con bajo contenido en grasa intramuscular mostraron mayores niveles de microorganismos, al igual que a los 3 días en refrigeración. La concentración de sal y la relación sal en magro no tuvieron una influencia significativa sobre los niveles de microorganismos, mientras que la a_w influyó en los niveles de micrococáceas en el jamón Serrano control y en los niveles de aerobios mesófilos, psicrotrofos y micrococáceas en el jamón Serrano tratado por APH. Los jamones con valores medios de a_w mostraron mayores niveles de aerobios mesófilos, psicrotrofos y micrococáceas.

El tratamiento de APH redujo significativamente los niveles de microorganismos, en 1,63 log ufc g^{-1} para aerobios mesofilos, en 1,71 log ufc g^{-1} para psicrotrofos, en 0,44 log ufc g^{-1} para bacterias lácticas, en 1,15 log ufc g^{-1} para micrococáceas y en 1,95 log ufc g^{-1} para mohos y levaduras. Después de 5 meses en refrigeración se observó una recuperación de la mayoría de los grupos microbianos hasta niveles similares a los de las muestras control, con la única excepción de las bacterias lácticas.

La microbiota del jamón Serrano a los 3 días en refrigeración estaba compuesta principalmente por las especies bacterianas *Staphylococcus equorum*, *S. succinus* y *Bacillus subtilis*, así como por las especies eucariotas *Penicillium commune*, *P. chrysogenum*, *Aspergillus fumigatus, Sclerotinia sclerotiorum*, *Eurotium athecium*, *Moniliella mellis*, *Debaryomyces hansenii* y *Candida glucosophila*. El tratamiento de APH produjo ligeros cambios en la población microbiana, puestos de manifiesto por la desaparición de las bandas de *B. subtilis* y la atenuación de las bandas de *E. athecium*.

11.4.2. Jamón Ibérico

Se identificaron 122 compuestos en la fracción volátil del jamón Ibérico a los 3 días en refrigeración: 10 ácidos, 18 alcoholes, 11 aldehídos, 18 cetonas, 10 ésteres, 13 alcanos, 23 compuestos bencénicos, 5 compuestos azufrados, 4 furanos, 5 furanonas, 3 pirazinas, y 2 compuestos misceláneos. En el jamón Ibérico después de 5 meses en refrigeración se identificaron 116 compuestos volátiles: 11 ácidos, 19 alcoholes, 13 aldehídos, 16 cetonas, 7 ésteres, 9 alcanos, 21 compuestos bencénicos, 5 compuestos azufrados, 4 furanos, 5 furanonas, 3 pirazinas y 3 compuestos misceláneos.

La composición química del jamón Ibérico a los 3 días en refrigeración afectó principalmente a algunos ácidos, alcoholes, aldehídos, cetonas, compuestos bencénicos, compuestos azufrados y furanonas. El contenido de grasa intramuscular fue el parámetro composicional que más influyó sobre la fracción volátil del jamón Ibérico a los 3 días en refrigeración. El contenido en grasa intramuscular

influyó significativamente en 20 compuestos volátiles. Los jamones con valores medios y altos de grasa intramuscular mostraron niveles elevados de compuestos procedentes de reacciones de oxidación lipídica (cetonas y compuestos bencénicos) y de compuestos procedentes de la alimentación de los animales (xilenos). La concentración de sal influyó significativamente en 2 compuestos volátiles (etanal y 2,3-pentanodiona), aunque solo en las muestras control. Los jamones con concentración baja de sal mostraron niveles elevados para ambos compuestos, cuya procedencia es presumiblemente la oxidación lipídica. La relación sal en magro no influyó significativamente en los niveles de ningún compuesto volátil, mientras que la a_w influyó significativamente en 5 compuestos volátiles. Los jamones con valores altos de a_w mostraron niveles elevados de compuestos procedentes del metabolismo microbiano (etanol).

La composición química del jamón Ibérico después de 5 meses en refrigeración afectó principalmente a algunos ácidos, alcoholes, aldehídos, alcanos y compuestos bencénicos. El contenido de grasa intramuscular y la concentración de sal fueron los parámetros composicionales que más influyeron sobre la fracción volátil del jamón Ibérico después de 5 meses en refrigeración. El contenido en grasa intramuscular influyó significativamente en los niveles de 5 compuestos volátiles, aunque ninguno de ellos se vio afectado en las muestras control y las tratadas por APH a la vez. La concentración de sal influyó significativamente en los niveles de 5 compuestos volátiles. Los jamones con valores medios de concentración de sal mostraron niveles elevados de compuestos presumiblemente procedentes del metabolismo microbiano (ácidos de cadena corta). La relación sal en magro y la a_w influyeron significativamente en los niveles de 4 compuestos volátiles respectivamente. Los jamones con valores medios y bajos de la relación sal en magro mostraron niveles elevados de compuestos presumiblemente procedentes del metabolismo microbiano (ácido acético y 3-metil-1-butanol) mientras que los jamones con valores bajos de la relación sal en magro mostraron niveles elevados de compuestos procedentes de la oxidación lipídica (etanal). Los jamones con valores

medios de a_w también mostraron elevados niveles de compuestos procedentes del metabolismo microbiano (ácido acético).

El tratamiento de APH influyó significativamente en los niveles de 35 de los 122 compuestos volátiles identificados en el jamón Ibérico a los 3 días en refrigeración. Cuatro compuestos (1 aldehído lineal, 1 alcano y 2 compuestos azufrados) alcanzaron mayores niveles en los jamones Ibéricos tratados por APH mientras que 31 compuestos (9 ácidos, 1 alcohol, 7 aldehídos, 3 esteres, 3 alcanos, 6 compuestos bencénicos y 2 compuestos azufrados) alcanzaron menores niveles en los jamones Ibéricos tratados por APH. En el jamón Ibérico después de 5 meses en refrigeración, el tratamiento de APH influyó significativamente en los niveles de 34 de los 116 compuestos volátiles identificados. Once compuestos (1 ácido, 2 alcoholes, 1 aldehído, 3 cetonas, 3 compuestos azufrados y 1 compuesto misceláneo) alcanzaron mayores niveles en los jamones Ibéricos tratados por APH y, por el contrario, 23 compuestos (3 ácidos, 3 alcoholes, 10 aldehídos, 4 esteres, 1 compuesto bencénico, 1 compuesto azufrado y 1 furanona) alcanzaron menores niveles en los jamones Ibéricos tratados por APH.

De los 122 compuestos identificados en la fracción volátil del jamón Ibérico a los 3 días en refrigeración y de los 116 compuestos identificados en la fracción volátil del jamón Ibérico después de 5 meses en refrigeración, 109 fueron identificados tanto a los 3 días como a los 5 meses de la refrigeración. Trece compuestos (1-hidroxi-2-butanona, 6-metil-2-heptanona, butanoato de etilo, 3-metilbutanoato de etilo, heptanoato de etilo, nonano, hexadecano, 1-hepteno, 2-octeno, *m*-etiltolueno, 2,4-dimetilfenol, feniletanol y pirrol) se identificaron solamente al principio de la refrigeración y 7 compuestos (ácido nonanoico, 2-etil-1-hexanol, butanal, decanal, acetofenona, éter etílico y 2-metil-1,3-tiazol) solo al final de la refrigeración. La suma de los niveles de los compuestos volátiles fue 5,1 % menor al final del periodo de refrigeración en las muestras control y 1,3 % menor en las muestras

tratadas por APH que al principio de la refrigeración. Sin embargo estas diferencias no fueron significativas ($P > 0,05$).

Los niveles totales de ácidos, alcoholes, cetonas, ésteres, compuestos azufrados, furanos, furanonas, pirazinas y compuestos misceláneos disminuyeron ($P < 0,05$) y los niveles totales de compuestos bencénicos aumentaron ($P < 0,05$) durante los 5 meses de refrigeración en las muestras control de jamón Ibérico. Los niveles totales de ácidos, alcoholes, cetonas, ésteres, furanos, furanonas y pirazinas disminuyeron ($P < 0,05$) y los niveles totales de compuestos bencénicos, compuestos azufrados y compuestos misceláneos aumentaron ($P < 0,05$) durante los 5 meses de refrigeración en las muestras de jamón Ibérico tratadas por APH. Respecto a los compuestos volátiles individuales, 64 compuestos resultaron afectados por el almacenamiento en refrigeración de las muestras control, 72 compuestos resultaron afectados por el almacenamiento en refrigeración de las muestras tratadas por APH y 61 compuestos resultaron afectados tanto en las muestras control como en las tratadas por APH. Los niveles de ácido hexanoico, etanol, 1-metoxi-2-propanol, 1-hexanol, etanal, 2-propanona, 1-hidroxi-2-butanona, 2,3-pentanodiona, 6-metil-2-heptanona, 2,3-octanodiona, acetato de etilo, butanoato de etilo, 3-metilbutanoato de etilo, hexanoato de etilo, heptanoato de etilo, octanoato de etilo, hexano, heptano, 1-hepteno, 2-octeno, nonano, hexadecano, 2,4-dimetilfenol, 4-etilfenol, 4-pentilfenol, fenilmetanol, 2-fenil-etanol, feniletanal, 1-fenil-propano, naftaleno, metanotiol, disulfuro de carbono, 2-butilfurano, 2(3h)-5-metildihidrofuranona, 2(3h)-5-butildihidrofuranona, metilpirazina, dimetilpirazina, 2,3,5-trimetilpirazina y pirrol disminuyeron durante la refrigeración mientras que los niveles de ácido octanoico, ácido nonanoico, 2-propanol, 1-octanol, 2-etil-1-hexanol, butanal, 2-metilpropanal, octanal, nonanal, decanal, ciclohexano, alcano ramificado I, alcano ramificado II, etilbenceno, 1-metiletil-benceno, *o*-etiltolueno, estireno, acetofenona, disulfuro de dimetilo, trisulfuro de dimetilo, éter etílico y 2-metiltiazol aumentaron durante la refrigeración. Tres compuestos volátiles resultaron afectados solamente en las muestras control: los niveles de 2-nonanona y benzonitrilo disminuyeron durante la refrigeración mientras que los niveles de 2-pentanol aumentaron durante la

refrigeración. Once compuestos volátiles resultaron afectados solamente en las muestras tratadas por APH: los niveles de 3-octanona, propanoato de etilo, 2-hidroxipropanoato de etilo, octano, trimetilbenceno, fenol, 4-metil-fenol, sulfuro de dimetilo y 2-pentilfurano disminuyeron durante la refrigeración mientras que los niveles de heptanal y *p*-xileno aumentaron durante la refrigeración.

La composición química del jamón Ibérico a los 3 días en refrigeración influyó significativamente sobre los niveles de psicrotrofos, micrococáceas y bacterias lácticas. La a_w fue el parámetro composicional que más influyó en los niveles de microorganismos en jamón Ibérico a los 3 días en refrigeración afectando significativamente a los niveles de psicrotrofos y micrococáceas, que eran más elevados en los jamones Ibéricos control con valores altos de a_w. La relación sal en magro influyó significativamente en los niveles de bacterias lácticas en los jamones Ibéricos tratados por APH, que eran más elevados en los jamones con baja relación sal en magro. Sin embargo, ni el contenido de grasa intramuscular ni la concentración de sal influyeron significativamente sobre ningún grupo microbiano.

La composición química del jamón Ibérico después de 5 meses en refrigeración influyó significativamente sobre los niveles de mohos y levaduras. El contenido de grasa intramuscular fue el único parámetro composicional que influyó significativamente en los niveles de microorganismos en jamón Ibérico, afectando a mohos y levaduras en los jamones Ibéricos tratados por APH. Los jamones con contenido intermedio de grasa intramuscular mostraron mayores niveles de mohos y levaduras.

El tratamiento de APH redujo significativamente los niveles de microorganismos en jamón Ibérico, con descensos de 0,53 log ufc g^{-1} para aerobios mesófilos, 1,02 log ufc g^{-1} para psicrotrofos, 1,34 ufc g^{-1} para bacterias lácticas, 0,93 log ufc g^{-1} para micrococáceas y 0,81 log ufc g^{-1} para mohos y levaduras. Al cabo de 5 meses en refrigeración, se observó en las muestras tratadas por APH una

cierta recuperación de los microorganismos aunque los niveles de todos los grupos microbianos estudiados se mantuvieron significativamente más bajos que los de las respectivas muestras control.

La microbiota del jamón Ibérico estaba compuesta mayoritariamente por bacterias del género *Staphylococcus* y levaduras del género *Debaryomyces*. Las especies mayoritarias fueron *S. equorum* y *D. hansenii*. Es de destacar la detección de aislados pertenecientes a los géneros *Tetragenococcus* y *Carnobacterium*, que no habían sido encontrados previamente en jamón curado.